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Improved management strategies and renewable sources for sustainable phosphorus crop fertilization

Michela Battisti

Supervisor:
Prof. Carlo Grignani
Co-supervisor:
Dr. Laura Zavattaro

Cycle Coordinators:
Prof. Eleonora Bonifacio
Prof. Domenico Bosco

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Abstract

Due to the scarcity of phosphorus (P) reserves, an efficient management of the P fertilization strategy is required to improve the agro-environmental sustainability of crop production. The application of an adequate amount of P relative to crop requirements and the nutrient recycling with wastes can help to achieve this aim. The aims of the research were:

- to describe the soil P availability dynamic over the long-period and its dependence from the P balance, the P source and the soil type;
- to quantify the benefits of localized starter fertilization with P or N, and their combination, in soils with different physical and chemical properties located in the same environment, in maize for grain systems;
- to evaluate the effect of starter NP fertilization at sowing in six long-term fertilization managements (mineral fertilizers or animal manures) and different initial soil P availabilities, in maize for grain;
- to quantify the effects of the adoption of strip-tillage, compared to plowing, and of localized fertilization at maize sowing with biogas raw digestate or mineral NP fertilizer, on the early development, growth, grain yield and sanitary traits of maize, in two different kinds of soils;
- to evaluate the P fertilizer value of sewage sludge ash for pasture production on soils that differ in phosphate buffering and phosphate sorption capacity.

The results highlighted that the accumulated P balance is a good and powerful predictor of soil Olsen P dynamics in the long term, making it possible to compare experiments that differ in P supply, crop, crop uptake and duration. The role that soil characteristics play in the response of soil Olsen P to different P supply sources was evident, in particular concerning the constant level achieved in the soil as soil cumulative P balance approached $-\infty$. The soil Olsen P response was also influenced by P source. Specifically, P sourced from farmyard manure increased the available P pool to a greater extent than either other organics (bovine slurry, commercial organic fertilizers, green manure) or inorganic

fertilizers, due to its high C/P. The NP starter fertilization at maize sowing is a crop practice that enhances the agronomic benefits of timely sowing through the improvement of crop early development, grain yield and qualitive and sanitary grain traits. The results showed that this practice determined benefits even in soils tested high in P availability where the soil Olsen P ranged from 20 to 36 mg kg^{-1}. These results were consistent with the results of field experiment where the maize plant response to the NP starter fertilization with an increasing of the crop P uptake following the increasing of the soil P status up to the critical value of 39 mg kg^{-1} of soil. However, the effects of the NP starter fertilization evaluated in mineral or manured systems showed that the benefits in terms of grain yield increase were observed in the mineral system, only, while no increase of production was recorded on the systems fertilized with bovine slurry or farmyard manure. These results highlighted the potential use of animal manures in substitution of mineral P fertilizers. Another study highlighted the suitability of the adoption of the strip-tillage technique in different soils, especially when conjugated with a proper starter fertilization management performed with digestate or diammonium phosphate. The deep injection of digestate showed moderate advantages on the early development of maize and allowed the same yield to be achieved as the mineral starter fertilization. The last study reports clear evidence that sewage sludge ash originated from the wastewater incineration can be used as a P fertilizer for crop and pasture growth. However, the study also indicates that the soil tests need to be recalibrated for use with sewage sludge ash, and point out a potential problem for interpretation when farmers use sewage sludge ash.

1. General introduction

A sustainable intensification of crop production is needed to fulfil the future demand for food and feed, while reducing the environmental impact of the agricultural sector and increasing the efficiency of invested resources (Foley et al., 2011). Although the technological progress of the last century has pushed to a rapid increase in agricultural productivity, it is now clear that this growth is no longer sustainable in that form. The environmental impact is enormous and is causing large-scale pollution of water and air, destruction of biodiversity and soil erosion (van der Werf and Petit, 2002). In this sense, inappropriate nutrient management has terrible consequences in a context of demand and population explosion, depletion of natural resources and climate change (Olesen and Bindi, 2002). Therefore, we must take a step forward in improving the nutrient management of all agricultural systems.

Recovery and reuse of nutrients contained in waste streams, such as animal manures, sewage sludge and food chain waste, can make an important contribution to improve the nutrient use efficiency and support the transition to a circular economy (Hidalgo et al., 2021). Furthermore, recovery and reuse of nutrients can play a key role in addressing some issues concerning the current nutrient supply:

- Food production to feed a growing population (Ashley et al., 2011);
- Agronomic and economic sustainability of farms (Cordell and White, 2013; Withers et al., 2020);
- Environmental pollution (Sarvajayakesavalu et al., 2018);
- Urgency to reduce and recycle wastes from the agri-food chain (Withers et al., 2020);
- Dependence of the agri-food system on finite, unsecured and non-renewable resources (Neset and Cordell, 2012).

In this context, the performance of agriculture has traditionally considered as successful farming capable of delivering large amounts of primary agricultural products and services. However, it is increasingly establishing a green perspective, that recognizes the impact of agriculture on water, air and soil quality, land use diversity, ecologies and climate change. The European Green Deal (COM 2019/640) sets out how to make Europe the first climate-neutral continent by 2050. The Farm to Fork Strategy (COM 2020/381) and the biodiversity strategy (COM 2020/380) both fix 2030 target for nutrients: reduce nutrient losses by at least 50% while ensuring no deterioration on soil fertility, and result in a reduction in mineral fertilizer use by at least 20%.

These goals will be achieved by implementing and enforcing the relevant environmental and climate legislation in full, applying balanced fertilization and sustainable nutrient management, and by managing nitrogen (N) and phosphorus (P) better throughout their lifecycle. To this end, the Commission will work with Member States to develop an Integrated Nutrient Management Action Plan in 2022 (COM 2021/1000), whose key aspects are: address nutrient pollution at its source, ensure more sustainable application of nutrients, and stimulate the markets for recovered nutrients and increase the sustainability of the livestock sector.

All political interventions should start from the scientific knowledge of key processes of the P cycle in agroecosystems (§1.1), and are also strongly influenced by international market of P sources (§1.2).

1.1. Phosphorus in the agro-ecosystem

P is an essential nutrient for all living organisms, even though it is much less abundant in plants compared to N and potassium, having a concentration of about one-fifth to one-tenth of that of N and K in plant dry matter (Roy et al., 2006). P is critical in the plant metabolism, as it represents a structural element of different cell organelles (e.g., phospholipid plasma membranes), macromolecules (e.g. DNA and RNA), and energy transfer compounds (e.g. ATP) involved in basic biochemical processes such as nutrient transport, respiration, photosynthesis, starch and cellulose synthesis (Hawkesford et al., 2012).

Mineral P is primarily conserved in soils and sediments (90%), while it is present in the atmosphere in limited amount as particulate (Pierzynski et al., 2005; Celi and Bonifacio, 2016). Soil P cycling, shown in Figure 1, is strongly affected by the nature of the inorganic and organic phases, form and extent of biological activity, chemical properties of the soil solution (e.g., pH, redox potential) and environmental factors like soil moisture and temperature (Pierzynski et al., 2005).

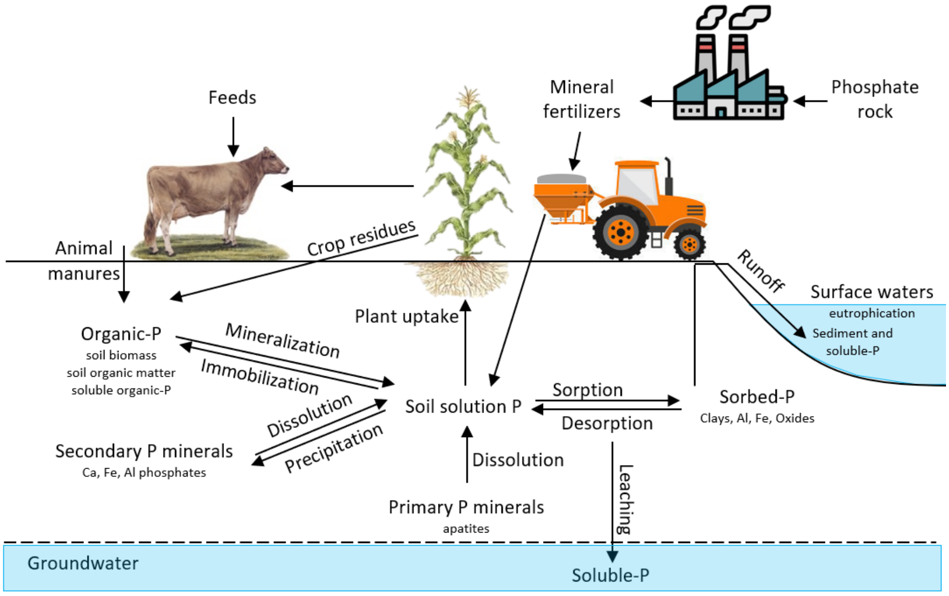

Figure 1. The soil P cycle (adapted from Pierzynski et al., 2005)

In most agricultural soils, 50-75 % of P is inorganic and originates from the weathering of apatite (Celi and Bonifacio, 2016). Inorganic P forms are dominated by hydrous sesquioxides, amorphous, and crystalline aluminum and iron compounds in acidic, noncalcareous soils, and by calcium compounds in alkaline, calcareous soils (Sharpley, 1995). This inorganic fraction may vary from 10% to 90% of the total P, depending on the climate and soil type (Sharpley, 1995). In Italy, the inorganic fraction prevails over the organic fraction in almost all agricultural soils (Celi and Bonifacio, 2016). Organic P forms include relatively labile phospholipids, inositols and fulvic acids, while more resistant forms are included in humic acids (Sharpley, 1995).

1.2. Global phosphorus fertilizers use

World agricultural use of inorganic P fertilizers (expressed as P_2O_5) strongly increased between 1961 and 2019 from about 10 million tonnes in 1961 to close to 45 million in 2019 (Figure 2, inset). The expansion of inorganic P use since the 1960s has been stronger in Asia (Figure 2). In 1961–1964 it represented less than 20% of the world total, compared to over 50% of the total, in 2015–2019. In the last five years, however, Asia showed no further growth in mineral P_2O_5 consumption. The Americas have remained, during most of the period 1961–2019, the region with the second highest levels of P_2O_5 use. Growth in the Americas, however, has been less intense than that observed in Asia. Data for Europe, in contrast, showed a strong reduction in agricultural use levels around 1990–1994, the time of the dissolution of the former USSR. Use of inorganic P_2O_5 in Europe remained quite flat overall since then, at about 4 million tonnes. This level represents about 9% the world total in 2015–2019.

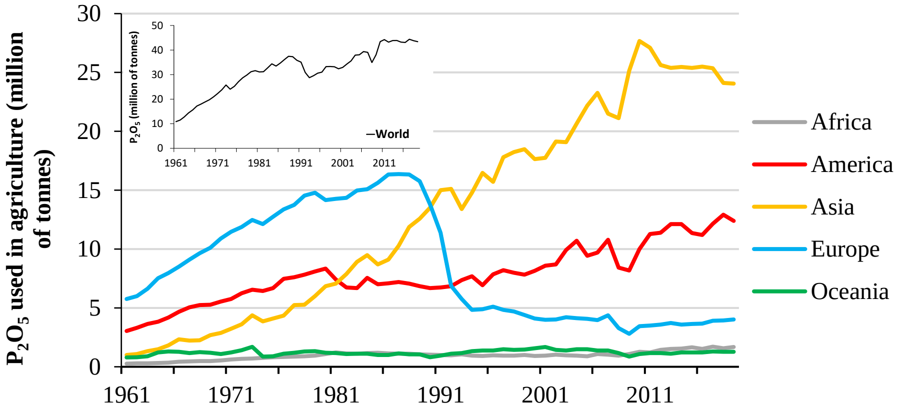

Figure 2. Agricultural use of inorganic P fertilizers (expressed as P_2O_5) in the different continents for the 1961-2019 period. Inset represents world consumption for the same period (data source: FAOSTAT, 2021).

The P balance, defined as the difference between input and output, provides an insight into links between agricultural P use, P losses to the environment, and the sustainable use of soil nutrient resources (Oenema et al., 2003). A persistent surplus indicates potential environmental problems, such as P runoff and leaching resulting in pollution of water and eutrophication of surface waters plus net soil nutrient accumulation. On the contrary, a persistent deficit can impair the resource sustainability of agriculture soil through soil degradation, or soil mining, resulting in declining fertility in areas under crop or forage production. Figure 3 is the result of the study of MacDonald et al. (2011), who calculated the P balance worldwide. It shows that there are many developing countries where the P deficit is significant. On the other hand, other regions (e.g., Central and Southern Europe, China, USA) are characterized by a remarkable P surplus. The P balance for the European Union was 1.2 kg per hectare per year in the period 2013-2015, much lower than the value of 3.9 kg per hectare per year in the period 2004-2006 (EUROSTAT, 2018).

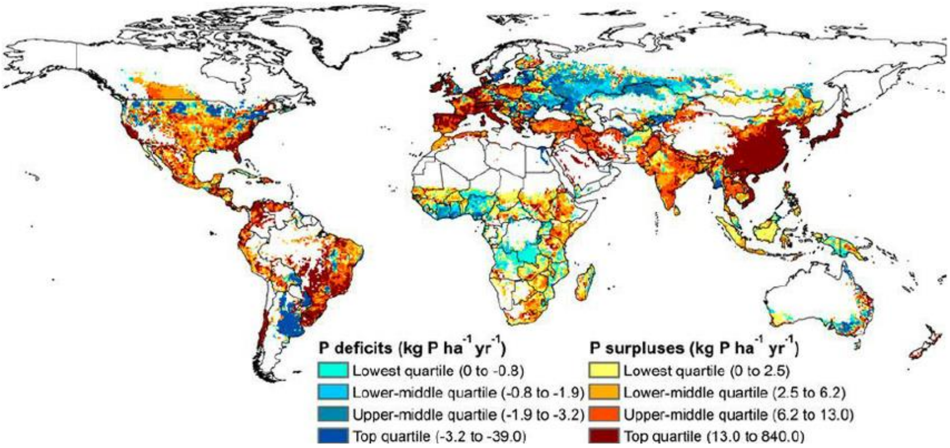

Figure 3. Global imbalances in phosphorous surpluses distribution (source: MacDonald et al., 2011)

According to a study by MacDonald et al. (2011), a total input of 23.8 million tonnes P year^{-1} to global croplands exceeded total removal by harvested crops (12.3 million tonnes P year^{-1}). Excessive P inputs can be due to an excessive use of mineral fertilizers, but also to a high animal density leading to manure inputs in excess to plant needs (Svanbäck et al., 2019). A surplus in the P balance will result in a P accumulation in soils (Ciampitti et al., 2011), and this will consequently increase the risk of P losses (Sharpley, 1995). The main environmental damage caused by P movement is the eutrophication of freshwater system (Figure 4) as it is normally the main limiting nutrient for blue-green algae growth (Cordell, 1999; Golterman and de Oude, 1991).

Figure 4. Eutrophic surface water (source: Michela Battisti, Canberra, ACT - Australia)

To date in the European countries more than half of water bodies are not in good ecological status (Figure 5), as required by the EU Water Framework Directive (2000/60/EC) (Poikane et al., 2019). Some Member States, like Belgium, Czech Republic, Denmark, Germany, Finland, the Netherlands, Hungary and Poland record bad water quality all around their territory and a systemic problem to manage nutrient losses from agriculture, while other countries, like Italy, Spain, Portugal and Romania have hot spots where pollution is not sufficiently addressed (COM 2021/1000).

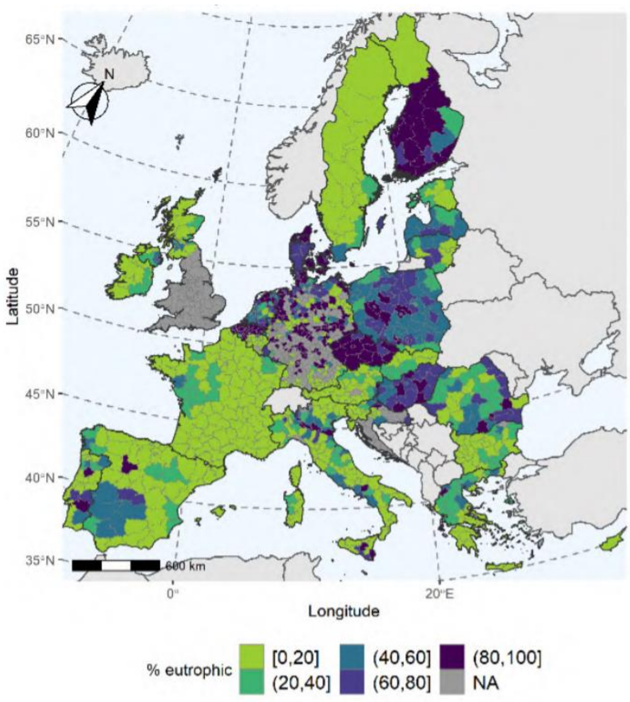

Figure 5. Eutrophic state for surface water by NUTS3 region (% of stations in eutrophic state) for the 2016-2019 period (from COM 2021/1000)

1.3. Phosphorus sources in agriculture

Phosphate rock is the main raw material used in the production of practically all phosphate fertilizers, although early sources of P used in agriculture production were primarily organic materials, i.e. bones and guano (Stewart et al., 2005). However, phosphate rock is a finite, non-renewable resource and most P mines are located in politically unstable regions, or the P produced is of poor quality due to high concentration of heavy metals (Scholz et al., 2013; Reijnders, 2014). Therefore, there has been an extensive discussion concerning the potential depletion of global P supplies. However, the immediacy of such a threat is arguable (Ulrich and Frossard, 2014; Sholtz and Wellmer, 2018) and it is not the primary reason for the imperative to improve the recycling of P. The prominent reasons are to: assist efforts to minimise P losses to the aquatic P cycle where P is a pollutant that is contributing to unprecedented eutrophication of waterways and aquatic ecosystem damage (Diaz and Rosenberg, 2008; Jarvie et al., 2013a, b; Campbell et al., 2017), to conserve the world's finite, high-grade P reserves for reasons of inter-generational justice, to reduce waste streams, to diversify the sources of P that can be used as fertiliser (Scholz and Wellmer, 2018), and to address consumer-led aspirations for more circular nutrient economies within agricultural systems (Stamm et al., 2021).

It is well established, as recalled in §1.1, that most of the P applied to agroecosystems in the form of mineral fertilizers, manures and wastes is retained in the soil as inorganic and organic P that is otherwise only sparingly available to plants (Condron et al., 2013). The fraction of applied soluble P that is immediately plant-available to the total amount of soluble P- applied as fertilizer in soil could be as high as 1:3 or less than 1:20, depending on fertilizer application rate, soil P-sorption properties and duration since fertilizer application (Hansen et al. 2002). In four long-term studies, for example, measured soluble plant-available P was <5% of total soil P for sites with no P fertilization and 6 - 87% for sites regularly fertilized with soluble P fertilizers or manure, depending on fertilization rates, soil type and texture (Hansen et al. 2002). The P accumulated in the soil is known as legacy P and represents large secondary global phosphorus source (Zhu et al., 2018). Singh et al. (2020) identified the disregard of legacy P inputs as one of the reasons for the poor performance of implemented best management practices in the P fertilization. The legacy P represents a large and global secondary source of P and could be used for crop nutrition during subsequent decades, substituting manufactured fertilizers and saving the limited phosphate rock reserves (Sattari et al., 2012).

Even though P resources are limited, only 5% of bio-waste is recycled for P production (Rosemarin et al., 2020). According to estimates, if more bio-waste was recycled, it could replace up to 30 % of non-organic fertilizers. Currently, the EU imports around 6 million tonnes of phosphates per year but could replace up to 30% of this total by extraction from sewage sludge, biodegradable waste, meat and bone meal or animal manures (Rosemarin et al., 2020). In line with these numbers, van Dijk et al. (2016) showed relatively little recycling of organic P sources in Europe, with the exception of manure in animal production, which is instead almost fully recycled. The use of animal manures as P source constitutes a key opportunity to save mineral fertilizers input, reduce the dependence on phosphate imports and losses and thus contribute to the nutrient recycling and the creation of a circular economy.

1.4. Methods to assess the P crop requirement

Continuous crop production requires an application of P from external sources in order to replenish the P taken up by crops and to avoid a decreasing, and finally too low soil availability of P. Historically, the grain yield increase for the major cereal crops have been observed at the world level since the beginning of the 20th century, which is associated with a 3.5-fold increase in the use of P fertilizers (Tilman et al., 2002). The crop application of a sufficient P amount is required to optimize production and farm income. In order to improve the P use efficiency, a more precise determination of when and how much fertilizer should be supplied to maximize the effect on the plant growth and on the crop yield, while minimizing the environmental impacts is urgently needed (Lemaire et al., 2021). Recommended fertilization and farmland nutrient management methods are mainly based on soil testing and crop yield responses (Mattila and Rajala, 2021). Because soil type, climate, and crop response vary considerably across the world, each country and sometimes even region has its own soil P test method and classification system (Jordan-Meille et al., 2012). Furthermore, some national fertilizer guidelines have used soil properties to adjust their recommendations. For example, the Italian P guidelines, based on the Olsen P soil test, use soil carbonates content and texture to modify the target soil test value as well as recommended fertilizer additions (MIPAF, 2021). On the other hand, Germany, that uses the Calcium Acetate–Lactate method, takes into account the stone, clay and soil organic matter contents (Jordan-Meille et al., 2012). In Australia, where the bicarbonate Colwell-P test is used, the phosphorus buffering index (PBI) is used to modify critical soil test values (Simpson et al., 2009).

The P balance calculated at the field scale is worldwide used to define the P fertilizer application rate. In general P inputs include P from mineral fertilizers, animal manures and other organic amendments, atmospheric deposition, seeds, while outputs are represented by the crop removals. Soil P tests are important in evaluating the soil nutrient availability for plant and the risk of soil P losses, and are therefore used to guide P management, fertilizer recommendation and regulatory program allowing the classification into fertility categories (Beegle, 2005; Sharpley and Tunney, 2000). For P, the "plant-based diagnosis" is often seen in opposition to the "soil-based approach" of predicting fertilizer P requirements (Lemaire et al., 2021).

The soil-surface P balance indicates the rate of P required to the crop to achieve the full potential yield. However, it does not allow the selection of the appropriate P fertilizer, as well as it does not indicate either the best timing or the best method of P application. The choice of P fertilizer to be used depends on several soil factors, climate conditions, crop characteristics, economics and secondary effects of fertilizers (Roy, 2006). Several studies have analyzed the long-term response of the soil plant available-P concentration in field following repeated additions of different rates of P and/or different types of P (i.e., mineral fertilizers, animal manures, composts, etc.) (Oehl et al., 2002; Shen et al., 2014; Singh et al., 2016), however, the effect of long-term past fertilization on plant growth and yield remains unclear. Based on all these considerations, it appears that long-term application of animal manures should be valorized and used efficiently to optimize P fertilization and reduce the application of mineral P. However, an effective nutrient management requires that nutrients will be available to the plant in adequate amounts according to the crop needs (Grant et al., 2001). Knowledge on short-term P availability after application of organic fertilizers (e.g., farmyard manure, slurry, sewage sludge, composts) provides an important basis to optimize fertilizer use with benefits both for the farmer and the environment.

1.5. Assessment of available P status of soils

The evaluation or assessment of soil fertility is perhaps the most basic decision-making tool for balanced and efficient nutrient management. Soil testing uses a range of conventional extraction methods involving different forms of acids (e.g., Bray and Mehlich), bicarbonate (e.g., Olsen and Colwell), salts (e.g., $CaCl_2$), or complexing agents, as well as pure water. Depending on the method used, quite different amounts of plant available P may be extracted from a given soil (Wuenscher et al., 2015). It is generally accepted that acid extractants are more appropriate for acid soils and bicarbonate extractants better suited to calcareous soils (Roberts and Johnston, 2015). Weak extractants such as water or sodium bicarbonate reflect mainly the intensity of supply, or in other words the concentration in the soil solution, while strong extractants primarily indicate the capacity of the soil to supply the nutrient to the soil solution, i.e. the buffer capacity (Marschner and Rengel, 2012). However, for P, a poorly mobile nutrient, conventional soil tests may overestimate the plant availability (Paris et al., 2004). Only a small fraction of soluble phosphate fertilizer that is applied to soil becomes available to plants over time. This is because soluble PO_4^{3-} is progressively converted to less soluble and less available P-forms by fixation processes such as precipitation and adsorption reactions in soil. The occurrence of these reactions depends on soil pH, concentrations of reactive Ca^{2+}, Fe^{3+} and Al^{3+}, type and amount of clays, and type and amount of organic matter (Hansen et al. 2002). Generally, the majority of the soil solution P is in the form of orthophosphate, as either HPO_4^{2-} (pH >7.2) or $H_2PO_4^{-}$ (pH 4.0-7.2) (Pierzynski et al., 2005). Phosporus is taken up from the soil solution by plant roots as orthophosphate ions, principally $H_2PO_4^{-}$ and to a lesser extent HPO_4^{2-}, except in calcareous and saline soils (Syers et al., 2008). In many agricultural systems, P is frequently the most limiting among macronutrients for optimal crop productivity (Grant et al., 2001; Hinsinger, 2001). Plant growth is markedly restricted under P deficiency, which delays growth, tillering, root development, and ripening. The deficiency symptoms usually start on older leaves (Roy et al., 2006). Plants become dark green to purplish in color (Figure 6).

Figure 6. Phosphorus deficiency symptoms in maize: purpling of the leaf margins starting on the older leaves (source: Michela Battisti)

A shortage of inorganic phosphate in the chloroplast reduces photosynthesis and respiration, while the chlorophyll concentration tends to increase and carbohydrates are accumulate favoring the anthocyanin synthesis, leading to dark green to purpling leaves. (Grant et al., 2001; Hawkesford et al., 2012). Because RNA synthesis is reduced, protein synthesis is also reduced. A decreased shoot/root ratio is a feature of P deficiency, as is the overall lower growth of tops (Roy et al., 2006). Plants respond to P deficiency by adaptations that maximize the likelihood of producing viable seed. Generally, P stress decreases the number of seeds produced more than the seed size (Grant et al., 2001).

1.6. Fertilization management to address the phosphorus issues

There is no one set of universal fertilizer best management practices, but rather they are site-specific and crop-specific varying from one region to another and one farmer to the next depending on soil type, climatic conditions, crop and cropping history, and management expertise (Roberts and Johnston, 2015).

However, there are many options to improve P management and avoid the typical problems associated with an inadequate fertilizer use. Work on improving the P use efficiency in crop production is a good starting point to increase the global food production, while making a rational use of external resources and minimizing the environmental pollution. One of these actions is to implement the "4Rs" nutrient management stewardship strategy whose base is to apply to the crop the right fertilizer or nutrient source, in the right amount, at the right time of application and using the right application method (IFA, 2009). Recently, Withers et al. (2015) outlined five key strategies that will deliver key policies, innovations, and measures to improve P efficiency, and reduce its P imports with potential benefits to global P resource management, environment, and biodiversity. These 5R strategies that include aspects from the technical management of the soil legacy P to the redesign of P use is the society and agro-food system are: re-align P inputs to meet only essential requirements, reduce P losses to water, recycle P in bioresources more effectively, recover P in wastes, and redefine P in food systems.

1.7. Open scientific challenges to address the P issues

Nowadays, the challenge is to manage the crop P fertilization to minimize environmental impacts while maintaining/optimizing production and farm profitability. The finite nature of the world's phosphate rock reserves also demands that P fertilizer be used as efficiently as possible, as P is a key nutrient that support global food security. Stamm et al. (2021) summarized the outcomes from the 9th International Phosphorus Workshop (IPW9) held in Zurich in July 2019 on how to move towards a sustainable P management. The key opportunities and challenges identified and prioritized to solve the P issues and develop sustainable solutions are related to:

- *P scarcity/optimizing P cycles*:
  a. Identify critical values of P in the soil to guide fertilization management;

b. Evaluate the role of soil legacy P as a source of P for crops and as a menace to the environment.

- *Sourcing P fertilizers:*
  c. Produce innovative fertilizers from recycled materials or renewable sources;
  d. Improve quality and fertilizer value of recycled materials;
  e. Develop new technologies and approaches for P recovery from waste.
- *Efficient P use in agroecosystems*:
  f. Understand the plant response to different amounts of fertilizer;
  g. Understand the plant response to different P sources;
  h. Assess the soil available P response to different fertilization schemes or net supplies;
  i. Identify the best fertilization practices that conjugate farm profitability and environmental concerns;
  j. Identify new biologically based approaches to mobilize the soil P;
  k. Select plants more efficient in P-acquisition based on genetic breeding.
- *Environmental P impacts:*
  l. Understand the role of the climate change on the P cycle.

1.8. Aims and objectives of the thesis

This work aims to contribute to points g, h, i, and to a minor extent to a and b reported in §1.7. The key questions include:

1. Is it possible to predict the soil Olsen P dynamics in the long-term through the accumulated P balance? Is it concurrently feasible to assess the influence of the type of soil and the P source?
2. How can P starter fertilization be more effectively managed in maize cropping system? Can maize starter fertilization with mineral P be avoided in farm that use animal manures?

3. Are there innovative strategies and sustainable alternatives to mineral P fertilization that reduce the consumption of raw materials and promote the P circular economy?

1.9. PhD thesis structure

The present thesis consists in five chapters, each one corresponding to scientific papers published or submitted to different international peer-reviewed journals, which report the main results of the research activities conducted at the Department of Agriculture, Forest, and Food Sciences (DiSAFA) - University of Torino under the supervision of Prof. Carlo Grignani and Dr. Laura Zavattaro and from the scientific collaboration established with Prof. Massimo Blandino and at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) - Agriculture and Food - Black Mountain Science and Innovation Park - Canberra (Australia) under the supervision of Dr. Rebecca E. Haling and Dr. Richard J. Simpson.

- Chapter 2: Battisti, M., Moretti, B., Sacco, D., Grignani, C., Zavattaro, L., 2022. Soil Olsen P response to different phosphorus fertilization strategies in long-term experiments in NW Italy. Soil Use and Management, 38, 549–563. https://doi.org/10.1111/sum.12701.
- Chapter 3: Blandino, M., Battisti, M., Vanara, F., Reyneri, A., 2022. The synergistic effect of nitrogen and phosphorus starter fertilization sub-surface banded at sowing on the early vigor, grain yield and quality of maize. European Journal of Agronomy, 137, 126509. https://doi.org/10.1016/j.eja.2022.126509
- Chapter 4: Battisti, M., Moretti, B., Blandino, M., Grignani, C., Zavattaro, L., 2022. Maize response to NP starter fertilization in mineral-fertilized or manured systems. Submitted to The Crop Journal.
- Chapter 5: Battisti, M., Zavattaro, L., Capo, L., Blandino, M., 2022. Maize response to localized starter fertilization with digestate under

different soil tillage methods. Submitted to European Journal of Agronomy.

- Chapter 6: Battisti, M., Simpson, R.J., Stefanski, A., Richardson, A.E., Haling, R.E., 2022. The phosphorus fertilizer value of sewage sludge ash for pasture production on soils that differ in phosphate buffering and phosphate sorption capacity. Accepted by Nutrient Cycling in Agroecosystems.

Chapter 2 describes the response of the soil available P (i.e., Olsen P) to different fertilization strategies, cropping systems and P balances in different types of soil using a data set compiled from three long-term experiments carried out in NW Italy. An empirical exponential model was proposed to describe the relationship between variables, and an agronomical interpretation of the optimized parameter values was also provided to set the basis for a unified interpretation of long-term dynamics of soil available P.

In the following chapters, the soil P availability in the short-term was assessed through the crop growth and yield responses.

A second study (Chapter 3) reported the effects of mineral P fertilisation applied alone or combined with N as starter fertilization at maize sowing, on the phenological development, biomass and yield production of the crop and also any potential quality and sanitary effects associated with earlier crop development. These effects were evaluated on soils that differed for the physical and chemical properties (e.g. texture and soil Olsen P concentration).

A third study (Chapter 4) aimed at evaluating the effect of long-term fertilization with mineral fertilizers or animal manures (i.e., bovine slurry and farmyard manure) on soil P availability in a continuous maize for grain cropping system. The hypothesis was that NP starter fertilization might be avoided in cropping systems characterized by positive accumulated P balance and different initial soil P availabilities.

Chapter 5 illustrates a field trial aimed at quantifying the effects of the adoption of strip-tillage or plowing, and of localized fertilization at sowing with biogas raw digestate or mineral NP fertilizer, on the early development, growth, yield and sanitary traits of maize, in two different kinds of soil. The underlying hypothesis was that a localized manure distribution could efficiently surrogate mineral NP fertilizers in promoting the early growth of maize, in particular in a context of reduced tillage techniques.

Chapter 6 refers to the research activity conducted during the research period abroad where the fertiliser value of an innovative P source derived from incinerated sewage sludge ash (SA) was compared with that of P supplied as superphosphate which is the dominant soluble-P fertiliser used for pasture production in Australia. Pasture (*Trifolium subterraneum* L.) growth responses were compared among three acid soils with contrasting P-buffering and P-sorption attributes. The ability of standard extractable soil-P tests to detect changes in P fertility after application of SA was also examined to understand how to measure soil fertility when SA was used as an alternative P fertiliser.

The obtained results are then discussed on a broader scale followed by general conclusions (Chapter 7), which summarize the potential use of improved management strategies and renewable sources to increase the phosphorus crop fertilization sustainability.

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2. Soil Olsen P response to different phosphorus fertilization strategies in long-term experiments in NW Italy

Authors: Michela Battisti[1], Barbara Moretti[1], Dario Sacco[1], Carlo Grignani[1], Laura Zavattaro[2*]

Affiliation:
[1] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy
[2] Department of Veterinary Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy

**Corresponding author:* Laura Zavattaro, Department of Veterinary Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy.
E-mail: laura.zavattaro@unito.it

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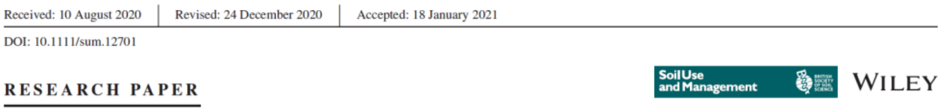

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RESEARCH PAPER

2.1. Abstract

Soil available P for crops, derived from P inputs and regulated by soil chemical, physical and biological factors, represents a small fraction of the total P content. Soil P balance (P input minus P output) is potentially a good descriptor of fluctuations in the available P pool, making it possible to compare experiments that differ in management and duration. Using a data set compiled from three long-term experiments in NW Italy that tested different P fertilization types (nil, mineral, organic and manures), cropping systems and P balances in different soils, this paper focused on (a) proposing an empirical exponential model to describe the relationship between variables and (b) providing an agronomical interpretation of optimized parameter values, to set the basis for a unified interpretation of long-term dynamics of soil plant-available P. The pooled data fit well in an exponential model that identified three factors supportive of an agronomic interpretation, two linked to soil attributes and one that differentiated fertilizer types. Soil characteristics were found to affect minimum soil Olsen P values in depletion and response rates with additions, while fertilizer type had a multiplier effect on cumulative P balance. Farmyard manure was shown to build the available P pool better than other organic sources (bovine slurry, commercial organic fertilizers, green manure) and inorganic fertilizers, owing to a high C/P. The exponential model provided a good theoretical basis, but further studies are needed to correlate parameter values to soil (e.g., carbonates, Al and Fe oxide content) and fertilizer (e.g., C:P, lignin:P) characteristics and to verify the model applicability to different conditions.

Keywords: cumulative P balance, exponential model, long-term experiments, P availability, P source

2.2. Introduction

Phosphorus (P), an essential nutrient for plant growth, is absorbed from the soil solution primarily in the form of orthophosphate ion (Jalali, 2009; Zhu et al., 2018). Even at high levels of soil total P, only a small fraction (<1%) of total P is actually available for crop uptake (Ohm et al., 2017; Zhu et al., 2018; Zicker et al., 2018). Soil chemical, physical and biological factors (Blake et al., 2000; Eichler-Löbermann et al., 2007) regulate available P in a variety of ways. Chemical factors that most influence available P include soil mineralogy, pH, organic matter, adsorption capacity and the presence of other chemicals (Aulakh et al., 2007; Hinsinger, 2001). In the case of pH, P deficiency can occur in either acidic or alkaline conditions because of P adsorption on Fe and Al oxides or P precipitation as Ca phosphates, respectively (Sharpley, 1995). Among the physical factors of soil that affect P availability, texture, temperature, aeration and soil moisture all play fundamental roles (Reijneveld et al., 2010; Schröder et al., 2015). Biological factors that most influence available P are root presence, crop residues, and bacteria and fungi (Ciampitti et al., 2011; Zhu et al., 2018). Each plant species has a different capacity to extract P from the soil through root exudation of organic acids, ions and phosphatases (Hinsinger, 2001). The amount of fertilizer certainly influences the amount of P that crops can utilize, but several authors, such as Edmeades (2003) and Shen et al. (2014), showed that available P also varies with the source of supplied P (e.g., organic or inorganic fertilizers). The sustainable use of phosphate-based fertilizers is a priority to make the agricultural system more environmentally sustainable (Tian et al., 2020) and promote the use of farm manures under correct agronomic principles (Bassanino et al., 2011). The implementation of fertilizer best management practices (4Rs: right nutrient source, right rate, right time and right place) aims to achieve the soil available P concentration able to enhance plant growth and to allow production goals of the farm (Roberts and Johnston, 2015; Simpson et al., 2011). Fertilizer-recommendation methods, either as Decision Support Tools or simple guidelines, require an estimate of the amount of P released from the soil (Jordan-

Meille et al., 2012; Reijneveld et al., 2010). There is no doubt that the best method to measure the plant-available P is to analyze the plant itself; there is in fact no agreement on which chemical extraction best mimics the root behaviour (Dyson and Conyers, 2013; Nawara et al., 2017). The most commonly employed methods to measure soil available P (Olsen, Mehlich-3 and Bray-1) correspond to a specific extractor type and pH value, and their reliability depends on the soil type (Reijneveld et al., 2010). The Olsen method (Olsen et al., 1954) is used for soils with mildly acid, neutral or alkaline pH, and it has wide international acceptance as an indicator of soil P fertility (Bierman and Rosen, 1994; Ma et al., 2009; Pagliari et al., 2010). Conversely, the Mehlich-3 method was developed for soils with acid to neutral pH (Zhang et al., 2014) and the Bray-1 method is indicated for soils with pH below 6.8 (Bray and Kurtz, 1945). The Olsen method is the reference standard in Italy. However, as Withers et al. (2007) reported, Olsen P averages only 2,7% of the total P in the 24 European soils that have been deeply characterized, which makes clear the importance of available soil P compared with measures of total soil P. Consequently, different estimation methods of soil available P may lead to very different recommendations of crop fertilizer requirements.

Common practice shows farmers often apply excess mineral P fertilizers because of the fear of low soil P availability, high-fixing soil capacities and low use efficiencies of mineral fertilizers (c. 20%) (Ciampitti et al., 2011; Roberts and Johnston, 2015; Zhu et al., 2018). P supply using organic fertilizers also exceeds crop requirements (Borda et al., 2011), because manure and slurry application guidelines are based on nitrogen, and the organic fertilizer N/P ratio is generally lower than that of plants. Then, because of the oversupply, soil P tends to accumulate over the long term (Alamgir et al., 2012; Ciampitti et al., 2011; Withers et al., 2001) and cause agricultural pollution through run-off and leaching (Lemercier et al., 2008; Messiga et al., 2010; Sharpley, 1995). Unfortunately, the amount of fertilizer to be supplied to crops is not explicitly ruled in any European-wide regulation; however, several European countries or regions have come to suggest specific limitations based on crop and/or soil characteristics to restrict P fertilization (Amery and Schoumans, 2014; Garske et al., 2020). In the Piedmont Region of northwest Italy, where this study is set, voluntary measures by the Rural Development Programme require that fertilization is withdrawn when the soil Olsen P concentration exceeds 25 mg kg^{-1} (Regione Piemonte, 2018). A pragmatic unique threshold concentration for all types of soils was set by the local legislation. Phosphorus accumulation in soils, termed legacy P, represents a large and global secondary source of P and could substitute manufactured fertilizers and improve preservation of limited phosphate rock reserves in the medium term (Rowe et al., 2016).

On the other hand, some locations may face severe P deficiency. When no fertilizer is added or when P supply < P removal, soil P availability declines, which may result in soil degradation and reduced capacity to sustain high crop yields (Ciampitti et al., 2011). Nevertheless, if the soil is rich due to past surpluses, it may take many years to reach such a significant depletion of soil available P (Sharpley, 1995).

A regional scale analysis of soils in Piedmont, NW Italy, pointed out that in the last decade, the soil Olsen P concentration increased in areas where most of the livestock farms are located, while it decreased in stockless areas, and in the paddy rice area (M. Battisti, unpublished data). Less recent data show that the fertilization management with both mineral and organic fertilizers provoked a P unbalance from −17 to 52 kg P ha^{-1} in the region (Bassanino et al., 2011). The same authors identified the highest P surplus in the lowland Piedmont Region where the stocking rate is high.

Several field-scale experiments have been conducted in the past decades to relate the plant-available soil P to fertilization practices including P accumulation, maintenance, and depletion in the long-term (Messiga et al., 2010). There is a general agreement on the need to explore variations over long periods, because of the slow reaction of soil (Morari et al., 2008; Van der Bom et al., 2017; Zicker et al., 2018), but the explanatory variable used was in some cases time (Dodd and

Mallarino, 2005; Johnston et al., 2016; Ma et al., 2009; Singh et al., 2016), in some other the net amount of P supplied, that is P balance. Soil surface P balance accumulated over time, in particular, has shown a great potentiality to compare cropping systems that vary in removal capacity and time duration. Many studies have found a positive correlation between soil available P and P balance (Blake et al., 2000; Colomb et al., 2007; Shen et al., 2014; Sucunza et al., 2018; Van der Bom et al., 2017). In the literature, most of the authors have explored a limited set of soil types, starting levels of soil available P, time spans, and fertilizer types and amounts, and in most cases, a simple linear relationship well described the relationship between P balance and plant-available soil P (Ciampitti et al., 2011; Messiga et al., 2010; Paris et al., 2004). The hypothesis that underlies a non-linear model is that processes that govern the available fraction of soil P are capable of two opposite responses, exerting a buffering effect at low availability rates, and a more than proportional increase, above a threshold for the soil capacity to hold them is reached (Ter Meulen, 1993). However, there is still a lack of consensus on which mathematical model should be used when a wider variability of P balance is explored and the linear model shows limited accordance with data. The model should be flexible, robust and possibly provide an interpretation of data that will allow an independent prediction of parameter values.

With this aim in mind, the research described here relied on three databases acquired from three long-term experiments that differed in soil characteristics, P fertilization type, cropping system, and P balance that were compared using the P balance accumulated over time. Using this data set, we intended to answer the following research questions:

- Can P accumulated balance predict soil Olsen P in the studied situations, spanning different cropping systems and soils, and a wide range of fertilization types and amounts, using a unique empirical model?
- Will the empirical model support an agronomical interpretation of optimized parameter values?

2.3. Materials and Methods

2.3.1. Experimental sites

This study analyzed data from three long-term experiments (LTEs) (Lombriasco, Cussanio and Tetto Frati) located in the Po River plain of the Piemonte Region in northwest Italy. Situated about 50 km apart (Figure 1), all locations experience sub-continental climate conditions with mean annual temperatures between 11.8 and 12.9°C and total annual rainfalls between 702 and 760 mm. Table 1 indicates the main chemical characteristics of the soil at initiation of the LTEs. The two soils at Lombriasco and Cussanio appear rather similar, while Tetto Frati has a deep calcareous and coarser-textured soil with low initial available P content. The soil organic matter (SOM) content was medium at all sites. Different treatments were considered at each site, whose details are reported in Table 2.

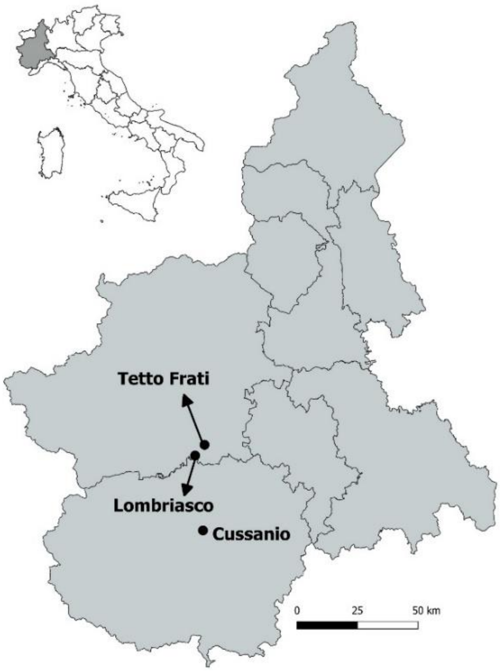

Figure 1. The location of the three long-term experiments in the Piedmont Region (NW Italy)

Table 1. Properties of the topsoil (0–30 cm) at the start of the LTE sites

	Lombriasco	**Cussanio**	**Tetto Frati**
Sand (%)	52.1	42.4	48.2
Silt (%)	35.3	47.5	44.3
Clay (%)	12.6	10.1	7.5
Soil pH_{H2O}[a]	6.5	6.5	8.1
$CaCO_3$ (%)[b]	0.0	0.0	4.7
Organic C (%)[c]	0.980	1.053	0.970
CEC ($cmol_{(+)}$ kg^{-1})[d]	9.0	11.0	10.1
Total N (%)[c]	0.109	0.107	0.126
Exchangeable K (mg kg^{-1})[e]	29.3	39.4	14.4
Bulk density (g cm^{-3})	1.61	1.59	1.42

[a] pH 1:2.5 w/v H_2O

[b] volumetric calcimeter method (Loeppert and Suarez, 1996).

[c] CHN elemental analyser (Flash EA 1112, Thermoquest; MIPAF, 2000).

[d] $BaCl_2$ extraction (Sumner and Miller, 1996).

[e] $BaCl_2$ extraction followed by AAS determination (Sumner and Miller, 1996).

Table 2. Treatments included in the study and details of the amounts of P supplied. Each treatment name is composed of 2-3 sections, separated by a hyphen. The first section includes an uppercase letter for the name of the site (L, C or T for Lombriasco, Cussanio and Tetto Frati), and a lowercase letter to indicate the time span (l, m or s for long, medium or short-term). The second section indicate the type of fertilizer, as detailed here. The third section, where present, indicates the starting soil P level (Lo for low, and Hi for high) in some of the treatments.

Site	**Time span**	**Fertilization strategy, and kg ha^{-1} yr^{-1} of P supplied**	**Initial soil Olsen P (mg kg^{-1})**	**Treatments**
Lombriasco (L)	1996-2016 (l)	Nil	49.0	**Ll-0**
Lombriasco (L)	2007-16 (m)	Nil	50.3 (Hi)	**Lm-0-Hi**
Lombriasco (L)	2007-16 (m)	Nil	27.8 (Lo)	**Lm-0-Lo**
Lombriasco (L)	1996-2007 (m)	Mineral, 35	60.2	**Lm-Min**
Lombriasco (L)	2007-16 (m)	Compost/digestate, 30	47.6 (Hi)	**Lm-Org-Hi**
Lombriasco (L)	2007-16 (m)	Compost/digestate, 30	26.9 (Lo)	**Lm-Org-Lo**
Cussanio (C)	2000-12 (m)	Nil	28.8	**Cm-0**
Cussanio (C)	2006-12 (s)	Nil	37.5	**Cs-0-Hi**
Cussanio (C)	2006-12 (s)	Nil	29.0	**Cs-0-Lo**
Cussanio (C)	2000-12 (m)	Farmyard manure, 42	38.3	**Cm-Fym**
Cussanio (C)	2000-12 (m)	Organic fertilizers, 39 + Green manure	18.7	**Cm-OG**
Cussanio (C)	2006-12 (s)	Farmyard manure, 39	38.3	**Cs-Fym**
Cussanio (C)	2006-12 (s)	Organic fertilizers, 32 + Green manure	34.7	**Cs-OG**
Tetto Frati (T)	1992-2012 (l)	Mineral, 60	n.d.	**Tl-Min**
Tetto Frati (T)	1992-2012 (l)	Bovine slurry, 25 and 51, + Mineral, 29	n.d.	**Tl-Slu**
Tetto Frati (T)	1992-2012 (l)	Farmyard manure, 46 and 90, + Mineral, 29	n.d.	**Tl-Fym**

2.3.1.1. Lombriasco

The Lombriasco site, located at 44°50′N, 7°38′E, contains soil classified as Typic Hapludalf, loamy sand, coarse-loamy, mixed, non-acid, mesic (Soil Survey Staff, 2014). The LTE had been managed as a grain commercial farm with winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soya bean (*Glycine max* (L.) Merr.), and maize cultivated under a 4-year crop rotation. All wheat cultivars were classified as hard (Cocchi et al., 2005), while all maize hybrids were in the FAO maturity group 500; soya bean varieties belonged to maturity group 1+. Each year, the wheat straw was removed and maize and soya bean residues were incorporated into the soil. Over the years, comparisons were made of three different treatments: tillage (ploughed vs. minimum tillage), fertilizer type (organic vs. mineral) and NPK total supply. This LTE was started in 1996 with large plots. At the end of this phase, each large plot had a different soil P content that was set as the initial value for a second experimental phase of the platform. To consider new treatment sets and various fertilization strategies to soils of varied initial P content, the design was in fact modified in 2007 to a split-plot design with four replicates. Mineral fertilizers were used before the LTE started. For further details, see Alluvione et al. (2011) and Moretti et al. (2020).

Three fertilization strategies were considered at the site: no P supply, moderate P mineral fertilization, and organic P fertilization using compost. The effect of no P supply was tested during two periods:

1. long-term period of 20 years (Ll-0);
2. medium-term period of 9 years on plots with two different initial soil Olsen P concentrations, high (Lm-0-Hi) and relatively low (Lm-0-Lo).

The effect of adding P supply was tested in the following circumstances:

1. medium-term period of 11 years on plots where mineral fertilizers were supplied as triple superphosphate (Lm-Min);
2. medium-term period of 9 years on plots at two initial soil Olsen P concentrations (Lm-Org-Hi and Lm-Org-Lo), both fertilized with composted municipal solid waste (MSW compost) or digestate.

2.3.1.2. Cussanio

The Cussanio site (44°34′N, 7°41′E) contained soil classified as Aquic Haplustept, coarse-loamy over loamy-skeletal, mixed, non-acid, mesic (Soil Survey Staff, 2014). The three-replicate experimental design was set in 2000 as big plots and then modified in 2006 into split plots and a different crop rotation.

Like at Lombriasco, the splitting of the original big plots in the second phase gave rise to a set of treatments applied to differentiated initial soil P levels. The original 3-year rotation of winter wheat –soya bean or field pea (*Pisum sativum* L.) –maize was also revised into a 4-year crop rotation of wheat and maize with 2 years of grass meadow. Maize and pulse varieties were similar to those used at Lombriasco, while wheat cultivars were of the soft class (Cocchi et al., 2005). The wheat straw was removed, while residues of maize and pulses were incorporated into the soil. Prior to the start of the experiment, mineral fertilizers were used only in a cereal production system. Further details are reported in Sacco et al. (2015).

This experiment allowed us to study the absence of P supply under two situations:

1. medium-term period of 12 years (Cm-0);
2. short-term period of 6 years on plots with two different initial soil Olsen P concentrations (Cs-0-Hi and Cs-0-Lo).

The P supply was tested under two scenarios:

1. medium-term period of 12 years on plots fertilized with farmyard manure (Cm-Fym) and on plots supplied with commercial organic fertilizers authorized for organic farming. This treatment also included a hairy vetch (*Vicia villosa* Roth) green manure (Cm-OG);
2. short-term period of 6 years on plots supplied with farmyard manure (Cs-Fym) and on plots fertilized with organic fertilizers, including hairy vetch green manure (Cs-OG).

2.3.1.3. Tetto Frati

The Tetto Frati site was located at 44°53′N, 7°41′E, where the soil is classified as Typic Udifluvent, coarse-loamy, mixed, mesic, calcareous (Soil Survey Staff, 2014). This LTE was established in 1992. The experimental design (randomized block with three replicates) compared four cropping systems: maize for silage, maize for grain with straw incorporation, silage maize + Italian ryegrass (*Lolium* *multiflorum* Lam.) double cropping and 4-year or 6-year rotation of silage maize and grass ley with three fertilizer management strategies described here:

1. constant mineral P application level as triple superphosphate (Tl-Min);
2. two rates of bovine slurry corresponding to 170 and 340 kg N ha^{-1}, where the amounts of applied P varied correspondingly, plus a mineral P contribution (Tl-Slu);
3. two rates of farmyard manure plus triple superphosphate, again based on fixed N dosed from manures (Tl-Fym).

Each of the considered fertilization managements spanned 20 years (1992–2012), details of which are reported in Zavattaro et al. (2016). All maize hybrids were chosen in the FAO maturity group 500.

2.3.2. P balance

The annual P soil surface balance was calculated as the difference between total P supply and P removal following the IRENA (Indicator Reporting on the Integration of Environmental Concerns into Agriculture Policy) method (EEA, 2006). Various P supply sources included mineral and organic fertilizers, farmyard manure, slurry or compost, while P in seeds and atmospheric deposits was considered as negligible (Messiga et al., 2010). Removals of P from the system included P contained in the harvested yield and in crop residues –if removed from the field. These values were calculated from dry matter yields and their P contents, assessed every year for all crops and added matrices. Accumulated P balance was calculated as the sum of annual P balances for the period between experiment start and soil sampling for Olsen P determination. The C:P ratio of supply sources was calculated by averaging available data of their compositions (not all years were available), weighted over the amounts distributed in OG treatments, where different sources were used.

2.3.3. Soil sampling

The soils were sampled periodically from the three sites to determine Olsen P. At Lombriasco, where experimental plots were 300 m^2 wide, sampling occurred every 3 years (i.e., six times in the 20-year long-term treatment and four times in the 9-year medium-term treatments). At Cussanio, where experimental plots were 300 m^2 wide, sampling occurred five times in the 12-year medium-term treatments and three times in the 6-year short-term treatments. Only in Tetto Frati (75-m^2 wide plots) did sampling occur just once; it was performed in 2012 after a 20-year long-term period. The sampling procedure was identical at each site in that three soil cores were taken from the first layer (0–30 cm) of each different treatment plot using a 7 cm diameter auger, and mixed to obtain a final sample of c. 1.5 kg. All soils were air-dried and sieved through a 2-mm mesh screen. Plant-available P was determined using the Olsen method (Olsen et al., 1954). To allow for a more generalizable relationship between variables, soil Olsen P concentration (mg kg^{-1}) was used in place of soil P content (kg ha^{-1}), which depends on soil bulk density.

2.3.4. Data analysis

The relationship between soil Olsen P and cumulative P balance was described on pooled treatments and sites using the exponential model (1):

$$OlsenP = a + b * e^{(c*PB)} \qquad [1]$$

where *PB* is the accumulated P balance observed in each treatment when Olsen P was measured, and a, b and c are fitting coefficients. Parameters a and c were set as specific for each soil. Parameter b was split into two additive components, one valid for all soils (b_0 parameter), and five components, specific for each of the types of supply used the experiments (b_{Min}, b_{Org}, b_{OG}, b_{Fym} and b_{Slu}). The initial soil P status was not included among explanatory factors because it was considered just a transient condition, originated by the past history and not a descriptor of each observed treatment. A matrix was built using all accumulated P balance and soil Olsen P data, setting dummy variables to include/not to include specific additive effects to the three parameters. A dummy variable is a binary variable that can take the value of 0 or 1. Three dummy variables described the type of soil for *a* and *c* parameters. Six dummy variables indicated five different types of P supply and one reference for unfertilized treatments. The following equation then represents the statistical model:

$$OlsenP = (L * a_L + C * a_C + T * a_T) + \left(b_0 + Min * b_{Min} + Org * b_{Org} + OG * b_{OG} + Fym * b_{Fym} + Slu * b_{Slu}\right) * e^{\left((L*c_L+C*c_C+T*c_T)*PB\right)} \qquad [2]$$

where parameters *a*, *b* and *c* were defined above and are detailed in Table 3, while *Min*, *Org*, *OG*, *Fym* and *Slu* are the amounts of P supplied in each treatment under a defined strategy, and *PB* is the accumulated P balance at the time *OlsenP* was measured. The field replicates were kept separated. The fitting procedure used the nls function in the R software (R Core Team, 2019) statistical package.

Table 3. List of factors and parameters included in the model. The explanation of treatment names is reported in Table 2.

Factor	Factor types	Parameter	Treatments
Soil	Lombriasco	a_L; c_L	Ll-0; Lm-0-Hi; Lm-0-Lo; Lm-Min; Lm-Org-Hi; Lm-Org-Lo
	Cussanio	a_C; c_C	Cm-0; Cs-0-Hi; Cs-0-Lo; Cm-OG; Cs-OG; Cm-Fym; Cs-Fym
	Tetto Frati	a_T; c_T	Tl-Min; Tl-Fym; Tl-Slu
Fertiliser type	Absence of P fertilization	b_0	Ll-0; Lm-0-Hi; Lm-0-Lo; Cm-0; Cs-0-Hi; Cs-0-Lo
	Mineral fertilizers	b_{Min}	Lm-Min Tl-Min
	Organic fertilizers	b_{Org}	Lm-Org-Hi; Lm-Org-Lo
	Organic fertilizers+Green manure	b_{OG}	Cm-OG; Cs-OG
	Farmyard manure	b_{Fym}	Cm-Fym; Cs-Fym Tl-Fym
	Bovine slurry	b_{Slu}	Tl-Slu

2.4. Results

2.4.1. Soil Olsen P response to zero P supply

Soil Olsen P responded to a negative cumulative P balance. In the absence of additions to the soil of P mineral and organic fertilizers, the negative net balance led to a progressively larger accumulated negative balance (deficit) that varied with the amount of annual P removed and experimental duration (Figure 2). The Ll-0 long-term treatment showed the largest negative P balance among the treatments considered (−581 kg P ha^{-1}, on average) after 20 years of observation, which was followed by Cm-0 (−292 kg P ha^{-1}, on average) after 12 years. The 9-year, medium-term treatments, Lm-0-Hi and Lm-0-Lo, exhibited P balances of about −245 kg P ha^{-1}. Treatments that went without a supply of P, which also had the shortest periods (6 years), yielded the least negative P values: Cs-0-Hi (−149 kg P ha^{-1}) and Cs-0-Lo (−162 kg P ha^{-1}).

The different cumulative negative balances caused variable effects on the dynamics of soil Olsen P. In one group of treatments, soil Olsen P fell as the cumulative P deficit grew. The resulting reductions for this group from the initial values were 17.3 mg kg^{-1}, 7.5 mg kg^{-1} and 6.0 mg kg^{-1} in Ll-0, Lm-0-Lo and Cs-0-Lo, respectively (Figure 2). A second group of treatments, including Lm-0-Hi and Cs-0-Hi, maintained soil Olsen P values in line with those from the beginning of the experiment. The Olsen P value was preserved despite their P balance deficits reached −256 kg P ha^{-1} (Lm-0-Hi) and −149 kg P ha^{-1} (Cs-0-Hi). The third treatment group, which contained Cm-0 only, produced an apparent two-step pattern; the soil available P initially reduced and then increased to a limited extent as the cumulative deficit rose.

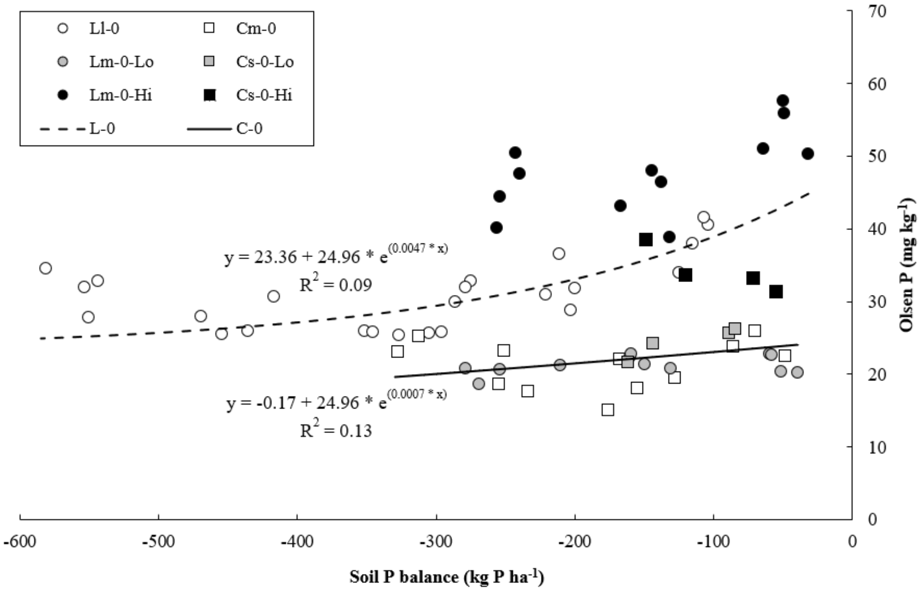

Figure 2. Relationship between soil Olsen P and accumulated P balance in the absence of P supply at Lombriasco in the long term (Ll-0) and in the medium term at high and low initial soil P availability (Lm-0-Hi and Lm-0-Lo, respectively); and at Cussanio in the medium term (Cm-0) and in the short term at high and low initial P availability (Cs-0-Hi and Cs-0-Lo, respectively). The interpolation lines used the parameters of equation 2 as reported in Table 4.

Typical asymptotic minimum values were detected at Lombriasco and Tetto Frati soils. The minimum at Lombriasco in Ll-0 was observed at 30 mg kg^{-1} of soil Olsen P after an initial cumulative P balance of −200 kg P ha, and Lm-0-Lo stabilized at 20 mg kg^{-1} at −278 kg P ha^{-1}. Similarly, at Cussanio, treatment Cm-0 exhibited a minimum at a rate of 20 mg kg^{-1}, or more, after a deficit of −150 kg P ha^{-1}.

2.4.2. Soil Olsen P response to mineral P supply

Two treatments - one each at Lombriasco and Tetto Frati - exhibited soil Olsen P responses to positive P balance supplied through mineral fertilizers (Figure 3). The Lm-Min treatment was managed with a fertilization supply oriented to match crop removal, such that it resulted in a soil P balance surplus of only 149 kg P ha^{-1} after 9 years. Conversely, accumulated P balances for different soils in the Tl-Min treatment ranged widely (88–794 kg P ha^{-1}) after 20 years. Furthermore, while both treatments exhibited a soil P balance surplus, the two sites displayed opposite trends. At Lombriasco, soil Olsen P clearly decreased as the accumulated P balance increased, whereas at Tetto Frati, Olsen P increased most impressively when the soil P balance increased widely.

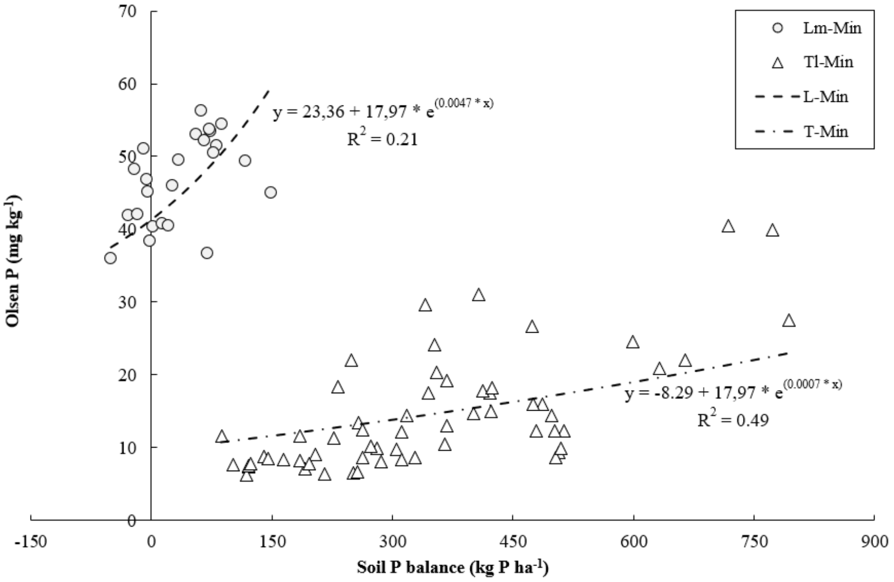

Figure 3. Relationship between soil Olsen P and soil P balance following repeated P supply using mineral fertilizers at Lombriasco (Lm-Min), and at Tetto Frati (Tl-Min). The interpolation lines used the parameters of equation 2 as reported in Table 4.

2.4.3. Soil Olsen P response to manure P supply

Four treatments, two each at Tetto Frati and Cussanio, allowed the soil Olsen P response to be studied following repetitive additions of bovine solid or liquid manure (Figure 4). The Tl-Fym farmyard manure fertilization management attained a P balance surplus above 1350 kg ha^{-1} after 20 years. On the contrary, the slurry fertilization management at Tl-Slu built a P balance that ranged from −254 kg P ha^{-1} to +780 kg P ha^{-1} according to the cropping system considered. The resulting variation depended both on the manure application rate and on the crop rotation. For example, at Cussanio, the P balance varied between 18 kg P ha^{-1} and 192 kg P ha^{-1}.

Phosphorus balance was shown to positively influence soil Olsen P concentration, as evidenced by results obtained from both the Tl-Fym and Tl-Slu treatments. In detail, soil Olsen P reached values from 32.0 mg kg^{-1} to 103.9 mg kg^{-1} and from 17.3 mg kg to 38.0 mg kg^{-1}, depending on the cropping system, after 20 years in the Tl-Fym and the Tl-Slu treatments, respectively. The low cumulative P surplus measured at Cussanio did not yield a clear relationship between soil Olsen P and soil P cumulative balance in either Cm-Fym or Cs-Fym treatments.

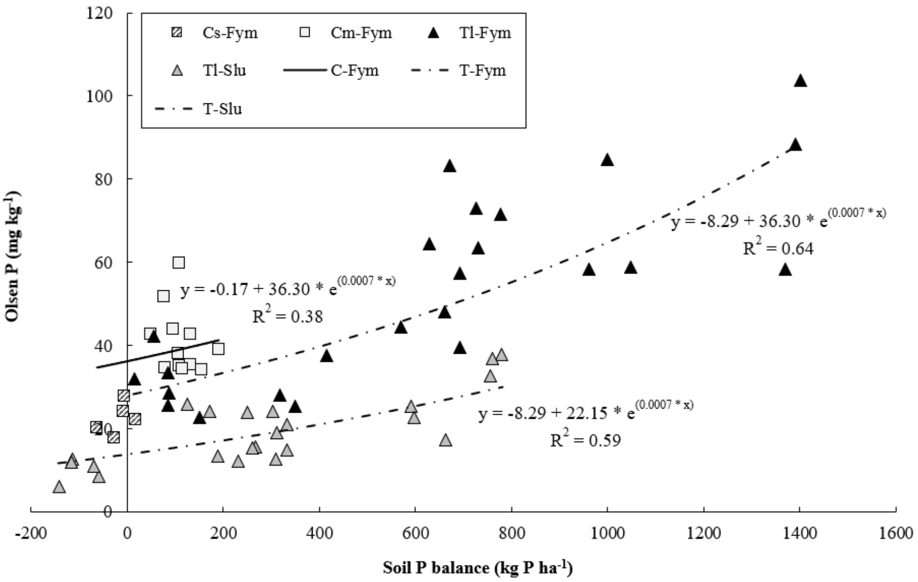

Figure 4. Relationship between soil Olsen P and soil P balance at Cussanio in the medium term and in the short term (Cm-Fym and Cs-Fym, respectively) following repeated additions of P supply using farmyard manure and at Tetto Frati in the long-term following repeated additions of P supply using farmyard manure plus mineral fertilizers and bovine slurry plus mineral fertilizers (Tl-Fym and Tl-Slu). The interpolation lines used the parameters of equation 2 as reported in Table 4.

2.4.4. Soil Olsen P response to other organic P fertilizer supplies

P fertilizer supplies Two treatments at Cussanio that differed for the duration and the initial soil Olsen P concentration showed the soil Olsen P response to annual addition of composted organic fertilizer coupled with green manure (Figure 5). The cumulative P balance produced a surplus of 380 kg P ha^{-1} in the Cm-OG treatment (medium term, 12 years long) and of 187 kg P ha^{-1} in the Cs-OG (short term, 6 years long). The soil Olsen P concentration remained constant in Cm-OG treatment (about 31 mg kg^{-1}) despite the high P balance ($p > 0.05$). Conversely, in the Cs-OG treatment starting from an initial concentration of 18.7 mg kg^{-1}, soil Olsen P increased with increasing cumulative P balance ($p < 0.05$). Two treatments at Lombriasco, equal for duration but different for the initial soil Olsen P concentration, gave insight to the soil Olsen P variation to composted municipal wastes and digestate yearly addition (Figure 5). The soil cumulative P balance ranged from about –50 kg P ha^{-1} to 100 kg P ha^{-1} at both treatments.

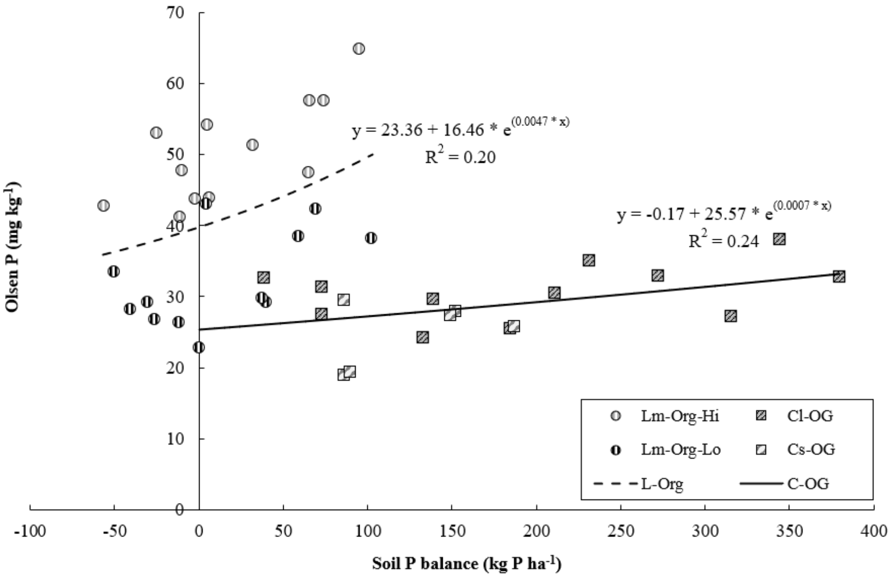

Figure 5. Relationship between soil Olsen P and soil P balance following repeated additions of P supply using organic fertilizers as MSW compost or digestate at Lombriasco in the medium term at high and low initial soil P availability (Lm-Org-Hi and Lm-Org-Lo, respectively) and green manure plus organic fertilizers authorized for organic farming at Cussanio in the long term and short term (Cl-OG and Cs-OG, respectively). The interpolation lines used the parameters of equation 2 as reported in Table 4.

2.4.5. Experimental model of soil Olsen P response to P cumulative balance

The fitted exponential equation had a NRMSE of 9.2% ($R^2 = 0.74$), which was considered very good. Optimized parameter values are reported in Table 4. Parameters *a* and *c* were specific for each experimental site, while parameter *b* was common to each group of P supply types (Table 3). Parameters *b* and *c* were mathematically correlated by a logarithmic function. They were also correlated through an agronomic interpretation, as matrices and soil responses interact in strict ways to determine available soil P concentration. Parameter *b* changed with the characteristics of fertilization, and in particular increased non-linearly with the C:P ratio of added P sources (Figure 6).

Parameters *a* varied among the sites and were approximately 23 mg P kg^{-1} at Lombriasco and 0 mg P kg^{-1} at both Cussanio and Tetto Frati (Table 4). The *b* value was higher for farmyard manure, minimum for mineral fertilizers and organic fertilizers (MSW compost and digestate), and intermediate for bovine slurry and green manure + organic fertilizers and for the absence of P supply. The *c* parameter was higher at Lombriasco and not significantly different from zero at Cussanio (Table 4).

Single R^2 values from each group of treatments ranged from 0.09 to 0.64. Although it is widely accepted that R^2 is not a good indicator of the goodness of fit in non-linear regression (e.g., Spiess and Neumeyer, 2010), it indicates a poorer model fit in depletion cases than in fertilized ones.

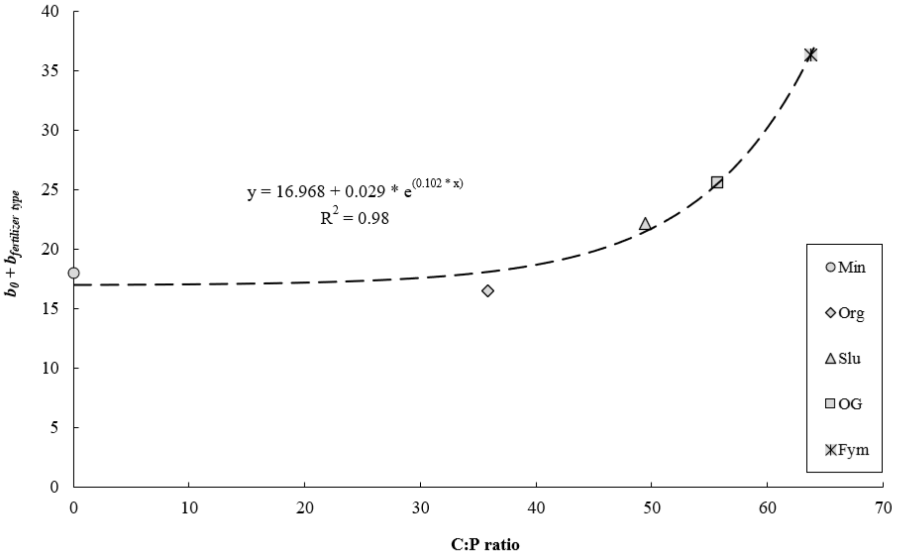

Figure 6. Relationship between the C:P ratio of different P sources and coefficient b of equation 2, calculated as b0 + bx, where x is the source of P as reported in Table 4.

Table 4. Parameter values and statistics of the nonlinear regression between cumulative P balance and soil Olsen P when all site and treatment data were pooled. S.E. = Standard Error, C.I. = confidence intervals at 2.5-97.5%, P(t) = probability of the t-test. For parameter a statistical differences are referred to the difference between a_L, a_C, and a_T and 0. For b parameter statistical differences are referred to the difference between b_{Min}, b_{Org}, b_{OG}, b_{Fym}, b_{Slu} and b_0. For c parameter, statistical differences are referred to the difference between c_L, c_C and c_T and 0.

Coeff.	**Estimate**	**S.E.**	**C.I.**	***P(t)***
a_L	23.36	3.96	(15.57 – 31.15)	*0.000*
a_C	-0.17	5.02	(-10.05 – 9.72)	*n.s.*
a_T	-8.29	5.26	(-18.65 – 2.06)	*n.s.*
b_0	24.96	5.06	(14.99 – 34.93)	*0.000*
b_{Min}	-6.99	3.27	(-13.42 – -0.56)	*0.033*
b_{Org}	-8.50	3.43	(-15.26 – -1.73)	*0.014*
b_{OG}	0.61	4.21	(-7.68 – 8.89)	*n.s.*
b_{Fym}	11.34	3.36	(4.73 – 17.95)	*0,.001*
b_{Slu}	-2.81	3.53	(-9.76 – 4.15)	*n.s.*
c_L	0.0047	0.0014	(0.0019 – 0.0076)	*0.001*
c_C	0.0007	0.0005	(-0.0002 – 0.0016)	*n.s.*
c_T	0.0007	0.0001	(0.0006 – 0.0009)	*0.000*

2.5. Discussion

The absence of P fertilization induced an initial drop in soil Olsen P, followed by a slow depletion until a constant level was reached. This observation agreed with reports by Ciampitti et al. (2011), in which suspension of P fertilization led to a rapid available P decrease in soils with high P levels and a gradual decline in soils with low initial P levels. Conversely, the accumulation of soil Olsen P was started slowly and grew quickly once the soil Olsen P concentration attained a critical value. Similarly, both organic and inorganic fertilizers caused soil available P to increase when applied to soils of high initial available P concentrations (Pote et al., 2003).

For medium-term scenarios, some authors found linear interpolation better for describing the effects on soil Olsen P of different fertilization managements, while an exponential one better described data pooled from different situations. A linear model means that the soil response to P fertilization is directly proportional to net additions, which may be the case under specific circumstances of soil immobilization processes. An agronomic interpretation of a linear model is quite simple –the intercept is the Olsen P value when the soil accumulated P balance is null (balanced fertilization) and at the beginning of the experiment; the slope represents the effect of each kg of P surplus or deficit on the soil Olsen P concentration.

It seems that when an experiment is limited to a single variable, a linear model is sufficient to describe the results, but when several soils and fertilization strategies (including severe depletion, heavy enrichment, and balanced fertilization) are studied, and initial soil P values are different, an exponential model function to cumulative P balance is a better descriptor of the results, both in depletion and in enrichment. Some authors used mixed models; for instance, Sucunza et al. (2018) applied a linear relationship to enrichment data and a simplified exponential model to depletion data.

The exponential model adopted here had three parameters (*a*, *c* and *b*), of which the former two were specific to soil type and the latter to P source. It had the advantage of describing a wide range of fertilization situations applied to different soils, with a good overall prediction capacity. In addition, through this model we could interpret some treatments in which a single linear or curvilinear interpolation would have failed, due to the limited variation in the P balance and high dispersion of the soil P status, such as in the Lm-Min and Cs-Fym treatments. Therefore, the exponential model can be used to interpret, generalize and forecast soil Olsen P trends following the adoption of different fertilization strategies, through the analysis of the optimized parameter values. Here, we analyze three fertilization strategies: suspension of fertilization, balanced supply and unbalanced supply. Then, we inspect the effect of soil and fertilizer types, also in interaction between them.

2.5.1. Long-term consequences of fertilization suspension

The suspension of P fertilization will cause a negative balance that will force the crop exploit legacy P from pools where most soil total P is immobilized (Sucunza et al., 2018). The soil Olsen P concentration will progressively reduce. The exponential model shows that when the accumulated P balance approaches - ∞, the Olsen P concentration will tend to an asymptotic value specific to each soil, that is coefficient a. It varied among the sites and was approximately 23 mg P kg^{-1} at Lombriasco and 0 mg P kg^{-1} at both Cussanio and Tetto Frati (Table 4). We recognize that P is a finite resource and that this makes parameter *a* more theoretical than real. Nonetheless, its value was not statistically different from zero at either Cussanio or Tetto Frati, while the value obtained at Lombriasco indicated that P should not be a growth-limiting factor at this site for a long time. The presence of an asymptotic level was also noticed by other authors (e.g., Ma et al., 2009; Messiga et al., 2015). Aulakh et al. (2007) and Zicker et al. (2018) also observed that the value of soil Olsen P without P supply first decreased, and then increased or remained constant. In our data set, two treatments (Ll-0 and Cm-0), after an initial depletion, showed a tendency to increase as a deficit grew. Unfortunately, our data were insufficient for analysis, but the phenomenon would benefit from further investigation.

2.5.2. Long-term consequences of a balanced fertilization

When P supply equals P removal in the long term, that is, P balance is close to zero, Olsen P will in the long-term approach $a + b$. This means that soil available P reaches an equilibrium based on both soil and fertilizer characteristics. Coefficient *b* stands for the P source effect, based on the well-established fact that variation in soil available P is influenced by the source of P (Van der Bom et al., 2017; Edmeades, 2003; Shen et al., 2014). In our model, the absence of P fertilization was set as a reference (b_0 parameter), and parameters were created for the supply type used in each experiment (b_{Min}, b_{Org}, b_{OG}, b_{Fym} and b_{Slu}). The *b* value quantified the relative effect of different sources on soil P availability and ranked them as follows: farmyard manure > bovine slurry and green manure + organic fertilizers = absence of P supply > mineral fertilizers and organic fertilizers (MSW compost and digestate) (Table 4). When the accumulated balance was null, the use of farmyard manure drove soil Olsen P concentration 11.34 mg kg^{-1} above that measured in the zero P supply (24.96 mg kg^{-1}), while mineral or organic fertilizer use resulted in a soil Olsen P concentration of 6.99 mg kg^{-1} and −8.50 mg kg^{-1} below that attained with no P supply (Table 4).

2.5.3. Long-term consequences of an unbalanced fertilization

The amount of soil available P for crops is regulated by a host of soil chemical, physical and biological factors (Ciampitti et al., 2011). Each of these aspects depends on soil type. An excessive fertilization will increase the soil reserves and affect the available pool size, whereas an insufficient level of supply will progressively deplete the available pool (Zhang et al., 2020). The exponential model tells us that the enrichment rate is different from the depletion rate on a linear scale with the P balance, and is indicated by a specific parameter – the *c* parameter – that is one of the components of the exponent in the function, a component that multiplies the P balance variable. The *c* parameter therefore represents the soil-dependent rate of decay or build-up of soil Olsen P. The higher the value of *c*, the more reactive is the soil to changes in the P balance. The model also informs us that the speed of the increase or decrease in Olsen P mainly depends on the soil characteristics. It was higher at Lombriasco than the other two soils (Table 4); therefore, Lombriasco showed a stronger response than Tetto Frati. This could be due to the fact that the calcareous Tetto Frati soil could buffer the Olsen P concentration to a larger extent (Liu et al., 2019), owing to the reaction of P with calcium carbonates, and according to the estimated values of *c*, the buffer capacity was nearly 7 times higher at Tetto Frati than at Lombriasco. The value of *c* did not differ significantly from zero at Cussanio. A value of zero sets to zero the exponent that includes the P balance; therefore, the accumulated

P balance was deemed not to influence the dynamics of soil Olsen P; only the source of P supply did so at Cussanio.

A fertilization strategy based on reduced or excessive supply will therefore affect the Olsen P soil pool to a greater extent at Lombriasco, to a minor extent at Tetto Frati and to a negligible extent at Cussanio. The Cussanio result may stem from soil characteristics or be artefact consequent to an absence of high supply cases in our data set.

2.5.4. Soil characteristics

Both *a* and *c* express the soil reactivity to changes in P balance, or in other words to soil P buffer capacity, a property that converts unavailable P pool into available P in depletion situations and vice versa in enrichment situations. Depending on soil characteristics, soil P buffer capacity can mask the relationship between soil Olsen P concentration and P balance, like at Cussanio (Ciampitti et al., 2011; Messiga et al., 2015; Zicker et al., 2018). The pH is the major factor that determines P adsorption on Fe and Al oxides under acidic conditions and P precipitation as Ca phosphates in neutral to alkaline soils (Hinsinger, 2001). Soil clay content also plays a role, as it is negatively correlated with changes in the status of soil available P (Cox, 1994). Therefore, the P buffer capacity of the young sandy-loam soil with an 8.1 pH and high $CaCO_3$ content at Tetto Frati was higher than that at Lombriasco, which had a loamy soil with a higher clay content, a pH of 6.2, and a high Fe and Al oxide content (data not shown). Data on the Fe and Al oxide contents at Cussanio were unavailable; however, the moderate clay content, greyish colour and neutral pH suggested the soil had a limited buffer capacity. Indeed, the Olsen P value at Cussanio reflected annual supply with fertilizer rather than a legacy of soil P. As the model was applied to three types of soils only, a further validation of this approach is needed.

2.5.5. Fertilizer types

The soil Olsen P response to P supply in the same soil differed for each source of P and was quantified by *b* parameter in the exponential model. Parameter *b* represents the amount of available P when the balance is at equilibrium, given a particular soil supply as represented by parameter *a*. Differences in the *b* parameter values related primarily to the quantity and quality of organic matter supplied by the different sources that released P after mineralization.

The *b* reference value (b_0) referred to the treatment with no supply of P and is theoretically linked to the transformation of unavailable into available P forms. The values of b_{Fym}, b_{Min} and b_{Org} differed significantly from b_0, but b_{Slu} and b_{OG} did not. That b_{Min} resulted as lower than b_0 indicated that the variation in Olsen P caused by mineral supply was less than the variation in the measure because of the absence of fertilization. This suggests that mineral fertilization, due to the fixing phenomenon, is limited in its ability to build the available P pool. Colomb et al. (2007) reported that 90–92% of P supplied with mineral fertilizers in excess of crop removal was transferred into a non-available pool. Some authors, such as Paris et al. (2004) and Zicker et al. (2018), recorded decreases in soil Olsen P, despite a surplus of mineral P supply.

The finding that b_{Org} was lower than b_0 showed that in the long term, compost fertilization did not benefit available P. However, in the medium term, the linear model results revealed that the compost supplied at Lombriasco provoked an increase in soil Olsen P, regardless of high or low initial concentration. This result agreed with reports by Evanylo et al. (2008) that soil available P increased with the application of compost, compared with unfertilized and mineral P treatments in the short term. The study by Moretti et al. (2020) at Lombriasco reveals that municipal solid waste (MSW) compost increased the most labile SOM fractions that are responsible to short-term P dynamics here outlined using the linear model, but did not increase the SOM stable fractions.

Values b_{OG} and b_{Slu} were similar to the b_0 value, suggesting that both green manure, even if added to composted commercial organic fertilizers, and bovine slurry resulted as similar to the treatment that received no P fertilizer. The resulting parameter at each treatment, that is the $b_0 + b_x$ value, where x is the type of P source, changed with the C:P ratio of the different matrices (Figure 6). The relationship was highly non-linear, and an exponential function in the same form as (1) well-fitted data, despite the paucity of cases does not allow for a further data analysis. However, the higher the C:P ratio, the higher the value of b, this meaning that organic C in the supply promoted the availability of added P. Damon et al. (2014) also observed that mineralization of fresh crop residue mineralization (green manure) increased with its C:P ratio, and argued that soil microorganisms are stimulated by the addition of matrices with a high C content, leading to a higher incorporation and subsequent mineralization of microbial biomass P, that at the end increases the plant-available fraction. In contrast, Jalali (2009) and Lupwayi et al. (2007) reported that the P released from plant residues or from organic P fertilizers was positively correlated with P concentration, but negatively correlated with the C:P and lignin:P ratio of the P source. The absence of an increased soil Olsen P concentration in the Cm-OG treatment, despite a surplus of c. 380 kg P ha^{-1} after 12 years, may be attributed to the low P amount released by mineralization of added matrices. As much as 80%–98% (Espinosa et al., 2017) of crop residue P can remain in organic forms and go undetected by the Olsen method. Not only does green manure fail to support available P pool build-up, but it also scarcely contributes to an increase in SOM content. Actually, it is more akin to a mineral fertilizer than to an organic one (Sacco et al., 2015). Bovine slurry also contains a large fraction of inorganic of P (Hansen et al., 2004), which caused it to fix quickly into non-available P form pools like P mineral fertilizers.

Only the farmyard manure P supply raised the concentration of soil Olsen P, which may be attributed to the P contained in organic compounds that ensured a slow, but efficient release of easily available P. It can also be mediated by increased phosphatases in the rhizosphere (Shen et al., 2014) or by development of microorganisms that foster stable SOM mineralization (Van der Bom et al., 2017). Manure mineralization involves formation of soluble organic phosphate compounds and organic acids that reduce P adsorption on Fe and Al oxides (in soils where this process prevails), and Ca-fixing compound solubilization in alkaline soils. Both processes lead to an increased P availability for crops (Jalali, 2009; Von Wandruszka, 2006; Zhu et al., 2018).

2.6. Conclusions

We confirmed that accumulated P balance is a good and powerful predictor of soil Olsen P dynamics in the long term, making it possible to compare experiments that differ in P supply, as well as in crop, crop uptake and duration. This study allowed us to answer positively to the research questions we posed.

An exponential model fitted well to soil Olsen P data of three long-term experiments spanning a wide range of fertilization management situations, using the accumulated P balance as the explanatory variable. Hence, we propose to apply this model to other field trials to verify its robustness to predict the long-term dynamics of soil available P response.

Pooling all experimental treatments together allowed to interpret situations that a single interpolation would have failed to relate to P balance. The exponential model supported a generalizable agronomic interpretation of all optimized parameters. The fundamental role that soil characteristics play in the response of soil Olsen P to different P supply sources was evident, in particular concerning the constant level achieved in the soil as soil cumulative P balance approached $-\infty$ (parameter *a*). This observation makes clear that extension agents, practitioners, but also P fertilizer-recommendation tools need to take different soil types into account when considering thresholds above which fertilization should be suspended. The soil Olsen P response was also influenced by P source (parameter *b*). Specifically, P sourced from farmyard manure increased the available P pool to a greater extent than either other organics (bovine slurry, commercial organic fertilizers, green manure) or inorganic fertilizers, due to its high C/P.

Additional studies on how to predict soil response from soil characteristics will give better insight into the capacity of a depleted soil to utilize legacy P to sustain crop uptake. Further investigation is also needed to forecast the behaviour of fertilizers and other organic matrices from some easily recoverable information, such as the C:P or lignin:P ratios.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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3. The synergistic effect of nitrogen and phosphorus starter fertilization sub-surface banded at sowing on the early vigor, grain yield and quality of maize

Authors: Massimo Blandino*, Michela Battisti, Francesca Vanara, Amedeo Reyneri

Affiliation:
Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy

**Corresponding author:* Massimo Blandino, Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy.
E-mail: massimo.blandino@unito.it

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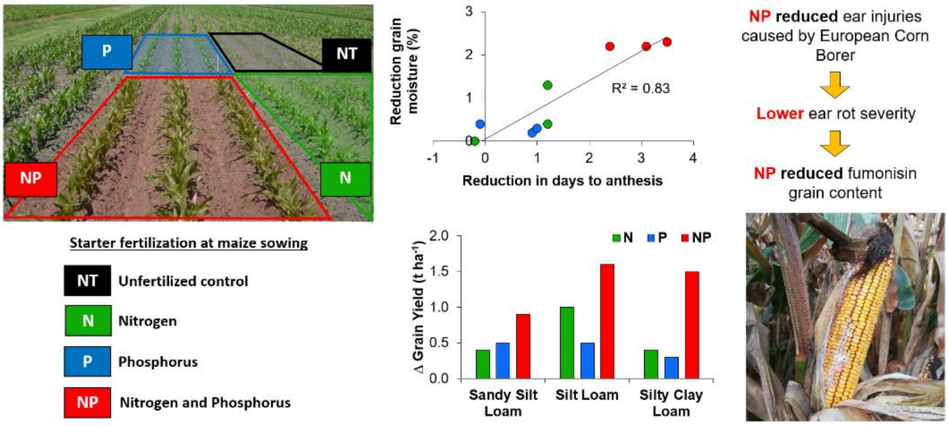
Graphical abstract



3.1. Abstract

Maize grain yields and profitability are enhanced in temperate growing areas as a result of an early sowing. However, cool conditions may constrain the uptake of nitrogen (N) and phosphorus (P) by the root system during the early and more susceptible growth stages. The application of starter fertilizers near the plant roots could ensure better N and P availability and improve early vigor of the crop. The aim of this study has been to quantify the overall benefits of a localized starter fertilization with N or P, or their combination, on maize (early development, grain yield, grain quality and sanitary traits).

A 4-year field experiment was conducted in NW Italy during the 2014-17 growing seasons. The trial compared the sub-surface placement of N, P and NP in bands close to the maize seed furrows, as a starter fertilization strategy, with unfertilized controls on sandy silt loam (SSL), silt loam (SL) and silty clay loam (SCL) soils. These soils were placed side by side, and were characterized by low, high and medium N and plant-available P contents, respectively.

The results showed that when N and P were applied together, the early vigor (plant height) and canopy development (NDVI values) of the maize were significantly enhanced, compared to both the controls and to the application of the single elements in all the soils. The positive growth responses to starter NP fertilization were combined with accelerated crop phenological development, so that anthesis occurred 2.4 to 3.1 days earlier. Thus, the grain yield was enhanced following the NP sub-surface placement (+7%, +15% and +11%, in SSL, SL and SCL, respectively), mainly due to an increase in the number of kernels per ear (+10%), while the grain moisture content was reduced, compared to the controls, in all the soils (-2.2%). The application of N or P alone did not consistently affect either the grain yield or the moisture content in any of the three soils. The starter fertilization strategy also affected both the qualitative and sanitary grain traits. NP and N both resulted in an increase in the grain hardness in the SL and SCL soils, but when P was applied alone, no significant effects were observed. The use of NP starter fertilization significantly reduced the ear injuries caused by

European Corn Borer in all three soils (-44%), and reduced ear rot severity in the SSL and SL soils and the fumonisin content (-72%) in the SL and SCL soils, compared to the untreated controls.

The synergistic effect of N and P applied as a sub-surface band at sowing had a consistent positive effect on the advancement of the flowering dates at different sites in different years, even in soils that have tested high to P or N. Thus, the application of NP starter fertilizer is a crop practice that should be considered in North Italy and for similar soils and environments.

Keywords: corn; flowering, soil texture; sub-surface placement in bands; mycotoxin

Abbreviations: ANOVA, analysis of variance; AUCDC, area under canopy development curve; CEC, cation-exchange capacity; DAP, diammonium phosphate; DAS, days after sowing; DM, dry matter; DON, deoxynivalenol; ECB, European Corn Borer; FBs, fumonisins B1 + B2; GDDs, growing degree days; GPC, Grain protein content; GS, growth stage; KSQ, kernels per square meter, N, nitrogen; NDVI, normalized difference vegetation index; P, phosphorus; REGWF, Ryan-Einot-Gabriel-Welsh F test; SCL, silty clay loam; SL, silt loam; SSL, sandy silt loam; TKW, thousand kernels weight, TME, total milling energy; TW, test weight.

3.2. Introduction

Maize (*Zea mays* L.) is the most important commodity crop worldwide, in terms of production, and its use is increasing in all food sectors (particularly for *gluten free* foods), for feed, industrial (starch industry) and energetic purposes (Shiferaw et al., 2011). Although this cereal has a high energy producing efficiency for food and feeds, its cultivation could have a marked impact on agro-environments because of its high demands for water and nutrients. In addition to environmental sustainability, the rising costs of crop inputs (fuel, fertilizers, pesticides, water) have reduced the economic competitiveness of this crop in the most intensive cropping systems and have led to demands for a new crop cultivation approach focused on practices with the highest efficiency and return of investments (Fischer et al., 2014).

Early sowing is essential in temperate growing areas in order to maximize the maize grain yields and profitability (Lauer et al. 1999). Late sowing tends to result in a lower grain yield due to the grain filling phase coinciding with a falling temperature and radiation levels and, consequently, a decline in kernel weight (Tsimba et al., 2013). Early sowing is recommended in dry areas or where there are crop irrigation limitations in order to allow the roots to develop to a depth before the soil water reserves become limited. Moreover, the sowing time may also play a significant role in reducing injury due to European Corn Borer (*Ostrinia nubilalis* Hüber; ECB) and fungal ear rot development (Blandino et al., 2009a). The quality benefits of maize with low mycotoxin concentrations are an important additional effect of an early sowing date (Blandino et al., 2009b).

However, in temperate growing areas, early sowing exposes a plant to more challenging conditions during the germination and leaf emergence stages, as a result of the lower temperatures coupled with more consistent and frequent rainfall. These conditions result in a high risk of pre- or post-emergence damping-off, insect injuries to the seedlings and weed competition (Miedema 1982). The delay in maize development caused by these stressful conditions reduces the benefits of an early sowing, which are primarily associated with a shorter time to anthesis and a grain yield increase (Van Roekel and Coulter, 2011; Blandino et al., 2017a).

Although maize breeding has delivered an increase in the tolerance to low temperatures in modern hybrids (Rodríguez et al., 2007; Peter et al., 2009), the occurrence of stress symptoms such as leaf redness may be detected during cool springs, as a result of colder soils, which slow mineralization processes and limit the uptake of nutrients by the root system (Niehues et al., 2004; Imran et al., 2013). Although many agricultural soils contain large amounts of total nitrogen (N) and phosphorus (P), often in excess of the plant requirements, the proportion of N and P available for plant uptake, particularly in the early vegetative stages, may be inadequate to satisfy the needs of a crop (Nkebiwe et al., 2016; Ohm et al., 2017).

Nitrogen is one of the key nutrients required to achieve the potential productivity of maize, and N availability will affect both grain yield and quality (Miao et al., 2007). An excess application of N fertilizer to a crop, particularly in the low uptake growth stages, increases the risk of N losses to the environment through leaching, surface runoff, denitrification and ammonia volatilization, thereby resulting in a waste of resources and pollution (Ma et al., 2019). The amount and timing of N applications, the soil permeability, and the quantity of rainfall can all affect N losses through leaching (Fageria and Baligar, 2005). Therefore, the nutrient supply needs to be synchronized with the plant uptake. Thus, in production situations in coarse-textured soils under medium-high rainfall regimes, the adoption of strategies designed to reduce the N amount applied before sowing, by maintaining a localized sub-surface application in bands close to seed furrows, can decrease the risk of N leaching (Sitthaphanit et al., 2009). Moreover, there are limits to how much N can be applied as a fertilizer banded close to seeds, due to the adverse effects on seed germination and young plants, as a result of a high salt concentration.

Phosphorus may be the main growth-limiting factor during the early stages of development of a crop (Grant et al., 2001) and this can occur in maize, especially after an early sowing or under cool or dry conditions (Schröder et al., 2015). Grant et al. (2001) highlighted the importance of supplying an adequate amount of available P during the early part of the growth season in order to avoid a P-deficiency. In order to optimize the P availability in a crop in rows, especially in the early growth stages, P fertilizers can be applied by placing it in the seeding zone as a starter fertilizer (Mallarino et al., 2011). Such application strategies meet the early plant demand by providing nutrients that are characterized by low solubility and a lack of mobility in close proximity to the small and developing root system. Bermudez and Mallarino (2002) recorded a high maize yield response to a starter fertilization on soils with a low P status. However, as a result of the concern about low soil P availability, high-fixing soil capacities, and a low use efficiency of P fertilizers (around 10-15%), farmers often apply excess amounts of mineral P fertilizers, thereby causing P accumulation in the soil (Withers et al., 2001; Zhu et al., 2018). This may increase the risk of P transfer to waters through leaching and runoff, and may consequently lead to freshwater eutrophication (Borda et al., 2011). Therefore, the need for additional P fertilizer applications to soils that have tested high to P availability is questionable (Schröder et al., 2015).

Farmers could apply starter fertilizers in bands close to maize seed furrows, thereby creating an area with a high concentration of nutrients near the plant roots, to ensure sufficient availability of N and P during the early growth stages. The response of maize to a starter fertilization is influenced by the seasonal conditions and soil texture, (Wortmann et al., 2006; Kaiser et al., 2016), and such an application is more profitable for late maturity hybrids and early sowing times (Kaiser et al., 2016). Although several studies have evaluated the benefits of NP-based starter fertilization on early maize development (Osborne, 2005; Jing et al., 2010; Jing et al., 2012; Ma et al., 2013; Ma et al., 2015; Kaiser et al., 2016), only a few have considered the relative benefits of the use of either single nutrients (N or P alone) or their combination in any detail. The advantage of applying a starter fertilizer containing either N or P, or both of these elements together, needs to be studied in soils with different textures and contents of these macronutrients, while taking into account their interaction with weather conditions in different growing seasons. Only a few field studies have been able to compare starter fertilizer effects in different soil types with contrasting physical and chemical properties under the same environmental conditions. If this work is conducted, it is typically carried out in a greenhouse, focusing on the early development stages (Kaiser and Rubin, 2013). In order to conduct a more complete evaluation of the benefits of starter N and P applications, there needs to be full growing season assessments of this application strategy. These studies need to consider the effects of such strategies on the phenological development, biomass and yield production of the crop and also any potential quality and sanitary effects associated with earlier crop development. Thus, the objective of the present study has been to quantify the overall benefits of localized starter fertilization with P or N, and their combination in the field, in soils with different physical and chemical properties located in the same environment.

3.3. Materials and Methods

3.3.1. Experimental site and treatments

The study was conducted at the University of Turin experimental station over four growing seasons (2014, 2015, 2016 and 2017). The site is located in the Po plain in the North-West of Italy at Carmagnola (44°53’ N, 7°41’ E; elevation 245 m), in an area where maize has traditionally been cultivated. The daily temperatures and precipitation were measured by a meteorological station in the experimental station.

The study was carried out on 3 large adjoining plots, each measuring 15 × 150 m and consisting of different types of soil, as far as the texture, and the physical and chemical properties are concerned:

- a sandy silt loam (SSL) soil (Typic Udifluvents, according to the USDA classification), which is the native soil of the experimental farm, and has a medium-low cation-exchange capacity (C.E.C.) and low N and plant-available P contents;
- a silt loam (SL) soil (Typic Dystrochrepts), which was transferred from the Riva presso Chieri site (44°59’ N, 7°52’ E), and has a medium C.E.C., a low N content and a high plant-available P content;
- a silty clay loam (SCL) soil (Typic Hapludalfs), which was transferred from the Sale site (44°58’ N, 8°48’ E), and has a high C.E.C, a medium N content and a medium P availability.

The SL and SCL soils were transferred in 1992 and they were placed to a depth of 0.60 m above the native soil. The soils were sampled at a depth of 0.30 m each growing season, using Eijkelkamp cylindrical augers just before crop sowing, and were analyzed for total N (using a CHN elemental analyzer, Flash EA 1112, Thermoquest) and available P (Olsen et al., 1954), according to MIPAF (2000). A 0-60 cm sample was collected from each soil and analyzed for the main physical and chemical parameters, as reported in Table 1.

Table 1. Main physical and chemical characteristics of the compared soils (sandy silt loam: SSL; silt loam: SL; silty clay loam: SCL) for the field experiments carried out in the 2014-2017 period

Parameters		**SSL**	**SL**	**SCL**
Sand (2 -0.05 mm)	g kg^{-1}	410	180	120
Silt (0.05 - 0.002 mm)	g kg^{-1}	540	670	560
Clay (< 0.002 mm)	g kg^{-1}	50	160	320
pH[1]		8.1	6.5	8.0
Total carbonate[2]	g kg^{-1}	20	-	12
Organic matter[3]	g kg^{-1}	13	11	16
C/N		8.8	9.1	8.2
Cation Exchange Capacity (C.E.C.)[4]	$cmol_{(+)}$ kg^{-1}	9.2	12.4	23.3
Exchangeable K[4]	mg kg^{-1}	39	49	86
Total N[3] (0-30 cm)				
2014	g kg^{-1}	0.80	0.69	1.22
2015	g kg^{-1}	0.94	0.72	1.12
2016	g kg^{-1}	0.95	0.72	1.25
2017	g kg^{-1}	1.07	0.87	1.27
Available P[5] (0 - 30 cm)				
2014	mg kg^{-1}	7	33	20
2015	mg kg^{-1}	9	36	25
2016	mg kg^{-1}	7	31	22
2017	mg kg^{-1}	8	31	24

Soil was sampled at a depth of 0-60 cm using Eijkelkamp cylindrical augers.
With the exception of the Total N and available P, the reported parameter values refer to a depth of 0-60 cm.
[1] pH 1:2.5 w/v H_2O
[2] volumetric calcimeter method (Loeppert and Suarez, 1996)
[3] CHN elemental analyser (Flash EA 1112, Thermoquest; MIPAF, 2000)
[4] BaCl2 extraction (Sumner and Miller, 1996)
[5] Bicarbonate-extractable P (Olsen et al., 1954)

Three different sub-surface starter fertilizers, placed in bands close to the maize seed furrows, were compared in each soil and for each growing season with untreated controls, without any starter fertilization:

- N fertilization, 27 kg N ha^{-1} was applied as ammonium nitrate (27% N w/w);
- P fertilization, 69 kg P_2O_5 ha^{-1} was applied as triple superphosphate (46% P_2O_5 w/w);

- NP fertilization, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} were applied as diammonium phosphate (DAP, 18 and 46% for N and P_2O_5, respectively w/w).

Diammonium phosphate is the starter fertilizer that is usually adopted in Northern Italy for banding at sowing. The treatments were assigned to experimental units in each soil using a completely randomized block design with four replicates. Each plot consisted of 4 rows 0.75 m apart, separated by two untreated buffer rows on either side; the plot length and the alleys between the plots were 12 and 1 m, respectively.

The starter fertilizers were placed at a distance of 5 cm from the side of the seed furrows using a calibrated granular dispenser applied to the planter (Monosem NG, Largeasse, France) at a depth of 10 cm from the soil surface. Studies were carried out each year on the commercial dent corn hybrid Pioneer P1543 (FAO maturity class 600; 130 days relative to maturity, Corteva Agriscience, Cremona, Italy), which is characterized by interesting kernel traits for the dry milling food supply chain (Blandino et al., 2013). The previous crop was maize each year. Mechanical sowing was carried out at the end of March – beginning of April, according to the recommended sowing time of the growing area, after a 0.3 m deep fall ploughing, followed by a suitable disk harrowing for each type of soil.

All the seeds were treated with a fludioxonil and metalaxil-m fungicide (Celest XL®, Syngenta Crop Protection S.p.A., Milan, Italy). Soil insect injuries were minimized by applying a tefluthrin non-systemic granular insecticide at sowing to the seed furrows (Force® 0.5%, Syngenta Crop Protection S.p.A.). Weed control was achieved by use of the pre-emergence herbicides mesotrione (0.15 kg AI ha^{-1}) S-metolachlor (1.25 kg AI ha^{-1}) and terbuthylazine (0.75 kg AI ha^{-1}) (Lumax®, Syngenta Crop Protection S.p.A., Milan, Italy). No foliar insecticide was applied during maize ripening to control European Corn Borer (ECB) or Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte).

Irrigation was carried out using overhead sprinklers, according to the conventional farm management system in use in the experimental area, to avoid any drought stress until physiological maturity (GS87). Before sowing, 100 kg ha^{-1} of K_2O was applied (as potassium chloride, 60% K_2O w/w), with no other N or P fertilizers applied except for the starter in bands close to the seed furrows. A total of 250 kg ha^{-1} of N (as urea, 46% N w/w) was applied at GS17 to all the soils and fertilization treatments as side-dressing fertilization.

The sowing and harvest dates, as well as the N side-dressing fertilization, are reported in Table 2 for each year.

3.3.2. Canopy development from the leaf to the tasseling growth stages

A hand-held optical sensing device, GreenSeekerTM® (Trimble©, Sunnyvale, California, the USA), was used to measure the normalized difference vegetation index (NDVI) during the vegetative stages. The instrument was held approximately 60 cm above each single maize row, and its effective spatial resolution was 0.75 m × the full length of the plot (12 m). This assessment was performed approximately every 7 days, in the two middle rows of each plot, starting from the three-leaf stage (GS13) and continuing until tassel emergence (GS55).

The NDVI measurement helped to quantify the development of the crop canopy throughout the season, since low values refer to bare soil, while high values are proportional to the maize biomass (Capo et al., 2020). The Area Under Canopy Development Curve (AUCDC) was calculated during the vegetative stage for each treatment, starting from the NDVI measurement for each observation date and using the following formula:

$$AUCDC = \sum_{i}^{n-1} \{[(R_i + R_{i+1})/2]\ (t_{i+1} - t_i)\}$$

where R is the NDVI value, t is the time of observation and n is the number of observations.

The plant growth rate was calculated as the average daily NDVI increase during the vegetative period, from the 4-leaf emergence stage to the middle of stem elongation.

The plant height was recorded at approximately the 7-leaf stage (GS17) and at stem elongation (GS33, approximately 3 detectable nodes) by measuring 20 consecutive randomly selected plants within the central two rows of each plot. Plant height was measured in centimeters from the ground level up to the collar of the tallest fully developed leaf (GS17), or from the ground level up to the tallest detectable node (GS33).

The number of days from sowing until the day when > 50% of the plants in the two central rows of each plot had reached the beginning of anthesis (GS62) was recorded. This parameter was expressed as days after sowing (DAS).

3.3.3. Grain yield and yield parameters

Ears were collected by hand at harvest maturity from 4.5 m^2 in the two central rows of each plot to quantify the grain yield and to obtain a representative sample. The harvesting was performed on the same day for all the starter fertilizer treatments and soils, when the grain moisture content was between 23 and 30%, according to the conventional harvesting in the growing areas. The collected ears from each plot were counted in order to record the density per square meter of the fully developed ears. The number of kernel rows and the number of kernels per row were also counted on 7 of these randomly selected and de-husked ears, and the theoretical amount of kernels per square meter (KSQ) was then calculated by multiplying the average number of kernels per ear by the number of ears m^{-2} (Testa et al., 2016). All the collected ears were shelled using a mechanical sheller. The kernels from each plot were mixed thoroughly to obtain a uniform sample, and grain moisture was analyzed using a Dickey-John GAC2100 grain analyzer (Auburn, IL, the USA). The grain yields were adjusted to a 14% moisture content. A 5 kg sub-sample was then taken and dried at 60°C for 72 hours, in order to reduce the kernel moisture content to 10%, for the qualitative analyses. Two hundred dry kernels were randomly collected and weighed to assess the thousand kernel weight (TKW).

3.3.4. Kernel quality traits

The test weight (TW) was determined on dry kernels by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp). The grain hardness was expressed as the total milling energy (TME), according to the procedure by Blandino et al. (2013) and conducted on grain at a 10% moisture content. A 20-g sample of kernels was ground, using a Culatti micro hammer mill (Labtech Essa®, Australia), fitted with a 2-mm aperture particle screen, at a speed of 2500 rpm when empty. The laboratory mill was equipped with a computerized data logging system to log the instantaneous electric power consumption during the milling test. The TME necessary to completely mill a 20-g kernel sample was determined from these data. This parameter was determined twice for each maize sample. The maize grains were ground using a ZM 200 Ultra Centrifugal Mill (Retsch GmbH, Haan, Germany), and the grain protein content (GPC; N × 6.00, on a dry matter basis, AACC 39-10) was determined according to AACC (2000).

3.3.5. Sanitary traits

A sub-sample of 20 ears was used to evaluate ECB and fungal ear rot severity at harvest, after removing the husk. ECB damage severity was calculated as the percentage of kernels per ear with injuries due to larva activity. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of the surfaces area of ear exhibiting visible kernel damage due to larva activity, according to the following schedule: 1 = no injuries, 2 = 1-5% of injuries, 3 = 6-10%; 4 = 11-20 %, 5 = 21-35%, 6 = 35-60%, 7 > 60% (Blandino et al., 2009c). Fungal ear rot severity was calculated as the percentage of kernels per ear with symptoms. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of the surface areas of ear exhibiting visible symptoms of the disease, according to the following schedule: 1 = no symptoms, 2 = 1-3 % of symptoms, 3 = 4-10%; 4 = 11-25 %, 5 = 26-50%, 6 = 51-75%, 7 > 75% (Blandino et al. 2009c). The ECB damage severity and ear rot severity scores were converted to percentages of ears exhibiting symptoms and each score was replaced with the mid-point of the interval.

A 4 kg representative sample of grain from each plot was milled. A representative sub-sample of the milled material was analyzed for mycotoxin concentration. Fumonisin B_1 and B_2 (FBs) and deoxynivalenol (DON) contaminations were analyzed using the ELISA method, by means of direct competitive immunoassays (RIDASCREEN® Fumonisin and RIDASCREEN® DON, R-Biopharm, Darmstadt, Germany), in accordance with the manufacturer's instructions. Briefly, both mycotoxins were extracted for 15 min by mechanically shaking, at 100 rpm, (shaker model M102-OS, MOM Instruments, Milan, Italy) 20 g samples with 100 ml of methanol/distilled water, (70:30, v/v) for FBs and with distilled water for DON, respectively. After extraction and filtration through Whatman® n.1 filters, 50 µl of diluted filtrate was used for the ELISA test. The optical density was measured at 450 nm using an ELISA 96-well plate reader (Das srl, Rome, Italy), and all the standard and sample solutions were analyzed in duplicate wells. The limit of detection (LOD) of the analytical method was set at 250 µg kg^{-1} and 37 µg kg^{-1} for FBs and DON, respectively.

3.3.6. Statistics

The Kolmogorov–Smirnov normality test and the Levene test were carried out to verify the normal distribution and homogeneity of variances. Analysis of the variance (ANOVA) was performed for early vigor parameters, date of flowering, grain moisture, grain yield and kernel qualitative traits such as ECB and ear rot severity and mycotoxin content, with the starter fertilization and the year as independent factors. ANOVA was carried out separately for each compared soil. Multiple comparison tests were performed, according to the Ryan-Einot-Gabriel-Welsh F (REGWF) test, on the treatment means. Statistical data analysis was carried out with the SPSS software package, version 24.0.

3.4. Results

3.4.1. Weather conditions

The four growing seasons showed different meteorological trends, as far as both rainfall and temperature (expressed as growing degree days, GDDs) are concerned (Table 2).

Table 2. Main agronomic information and cumulative monthly rainfall and growing degree days (GDDs) during the maize crop cycle in the 2014-2017 period at Carmagnola (North Italy).

Agronomic information	**2014**	**2015**	**2016**	**2017**
Sowing date	March, 20	April, 7	March, 23	March, 20
N side dressing fertilization date	May, 23	May, 20	May, 20	May, 19
harvesting date	September, 29	September, 1	September, 7	August, 31
		Rainfall (mm)		
Month	**2014**	**2015**	**2016**	**2017**
March	67	115	51	53
April	47	66	41	42
May	121	46	124	52
June	125	60	23	113
July	204	1	79	64
August	37	123	11	16
September	62	40	37	51
March - September	663	452	366	390
Sowing – 7 leaf stage	112	127	171	141
		GDDs (°C-day)[a]		
Month	**2014**	**2015**	**2016**	**2017**
March	110	80	77	126
April	163	161	158	160
May	224	264	218	253
June	342	354	332	359
July	359	437	403	394
August	357	371	378	397
September	282	245	304	242
March - September	1837	1911	1869	1931
Sowing – 7 leaf stage	272	377	259	261

[a] Accumulated growing degree days for each month using a 10°C base

The 2014 year had the highest rainfall, in particular during flowering (July), and it was characterized by cooler weather and the lowest GDDs during all the ripening stages. This led to harvest taking place at the end of September in 2014. Conversely, the 2015, 2016 and 2017 years had drier and warmer conditions during ripening and, as a result, the harvest was carried out at the beginning of September. July was the driest and warmest month in 2015, while rainfall was more evenly distributed in the other months. The coolest conditions in the spring months were observed in 2016, with a high amount of rainfall in May, while the temperatures were high and little rainfall was observed from flowering onwards. As far as the meteorological trend in the 50 days after sowing is concerned, the GDD were higher in 2015, as a result of a later sowing time. In this period, the temperatures in 2014 were similar to those in 2016 and 2017, but there was less rainfall.

3.4.2. Early vigor

The starter fertilization banded at sowing affected the plant development during the vegetative stages. The NDVI development of the respective fertilization treatments in the different soils is shown in Figure 1, considering the 2017 experiment as a representative example of the NDVI curve for the other considered years. Since the plant density was not different between treatments, lower NDVI values were related to a low plant development (vigor). It is possible to observe, from the reported curves, that maize growth in all the soils was faster with NP starter fertilization, with banded applications of only N or P resulted in an intermediate effect. The maize growth rate during the vegetative stage, expressed as the daily NDVI increase, was on average 0.0120, 0.0123, 0,0133 and 0.0145 NDVI d^{-1}, considering all the soils and years, for the controls, N, P and NP starter fertilization, respectively (data not shown).

Differences in plant vigor were progressively detected by means of the NDVI index in each soil and for each growing season, from the three leaf stage (GS13) to tassel emergence (GS55), and was expressed by means of the AUCDC index

(Table 3). The starter NP fertilization at sowing resulted in a significant (*P* <0.001) increase in AUCDC in all the soils, compared to the untreated controls or to the sub-surface banded fertilization with only N or P (Table 3). The NP fertilization on average increased this vegetative index by 19%, 20% and 18% in the SSL, SL and SCL soils, respectively, compared to the controls. N starter fertilization had a significantly higher AUCDC than the controls in the SL (+7%) and SCL (+8%) soils, while a significant benefit was reported for P banded application at sowing (+7%) for the SSL soil. Furthermore, a significant fertilization × year interaction was reported for all the soils. The NP treatment significantly increased the AUCDC for all the conditions (soils × years), compared to the untreated controls, with the only exception being the experiment carried out in the SCL soil in the 2014 growing season (data not shown). No significant increase in this vegetative index was observed for the N or P treatments in 2014, compared to the controls. Conversely, the banded P application led to a significant increase in AUCDC in the SSL soil (2015, 2016 and 2017) and in SL (2015), while the N starter fertilization alone resulted in an early crop development in SL (2015 and 2016), SSL (2017) and SCL (2016).

The plant development results, summarized by the AUCDC index for all the vegetative growth stages, were consistent with the height of the plants measured at GS17 and GS33. Compared to the control, NP fertilization significantly increased the plant height at the leaf emergence stage by 28%, 39% and 55% in the SSL, SL and SCL soils, respectively. The NP treatment led to an increase in plant height at the stem elongation stage of 91%, 63% and 68% in the SSL, SL and SCL soils, respectively. P-based starter fertilization only resulted in a greater plant height than the control for both of the considered GSs in SSL, while sub-surface banded N fertilizer alone significantly increased plant vigor in both the SL and SCL soils.

The interaction between starter fertilization and year was never significant for the plant height parameter in the SSL soil at either GS, or for SCL at GS33. Conversely, a significant fertilization × year interaction was reported in the SL soil, where, according to the AUCDC results, N starter fertilization only led to a significantly more rapid development of the plants in 2015 and 2016 (data not shown).

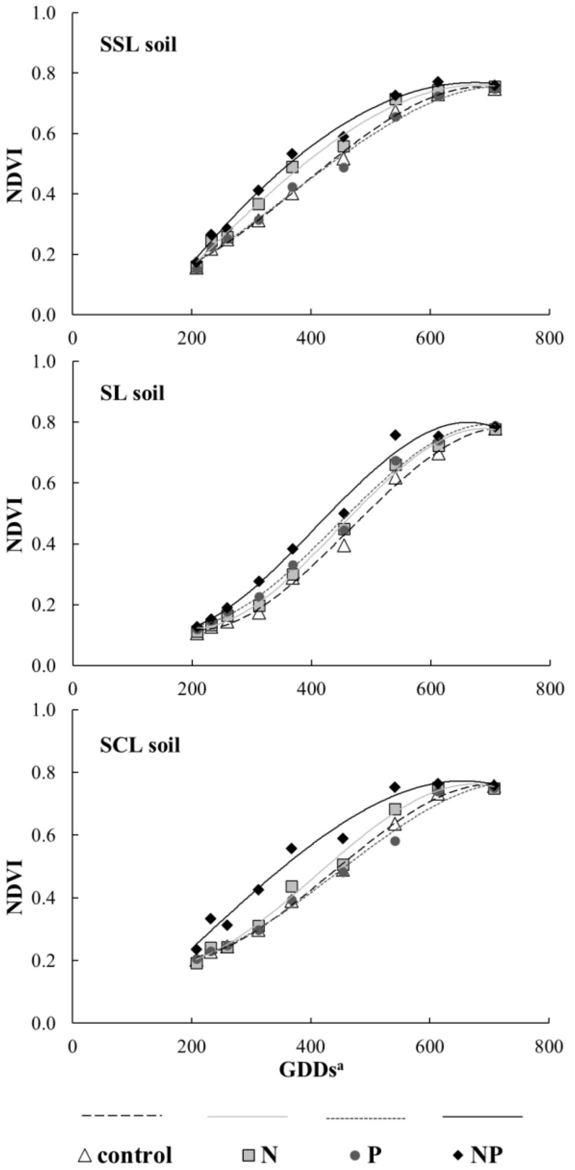


Figure 1. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on the maize canopy development, expressed as NDVI from the 3 leaf stage to tasseling; field experiments carried out in soil with different textures in 2017 at Carmagnola (Italy). SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

Table 3. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on the early vigor of maize, expressed as the area under the canopy development curve (AUCDC) and the plant height at the leaf emergence (GS17) and stem elongation (GS33) stages for field experiments carried out on soils with different textures in the 2014-2017 period[a].

Factor	Source of variation	AUCDC			Plant height GS17 (cm)			Plant height GS33 (cm)		
		SSL	**SL**	**SCL**	**SSL**	**SL**	**SCL**	**SSL**	**SL**	**SCL**
Starter fertilization (SF)[b]	control	22.3 d	25.2 d	21.5 c	19.7 c	23.5 d	17.4 c	31.6 c	47.1 c	35.2 c
	N	23.6 c	26.9 b	23.3 b	20.9 bc	27.0 b	21.4 b	37.6 b	59.1 b	45.3 b
	P	24.5 b	26.2 c	21.3 c	21.9 b	25.1 c	16.6 c	39.2 b	50.8 c	37.5 c
	NP	27.2 a	30.2 a	25.4 a	25.3 a	32.5 a	27.1 a	60.5 a	76.8 a	59.1 a
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Year (Y)[c]	2014	25.5 a	27.7 b	20.6 b	19.6 b	24.4 c	14.2 c	41.2 b	57.7 b	37.3 c
	2015	25.3 a	28.6 a	25.4 a	28.6 a	36.6 a	30.5 c	63.3 a	96.1 a	77.0 a
	2016	25.5 a	26.5 c	19.8 b	29.0 a	32.0 b	22.0 b	38.6 b	38.1 c	16.8 d
	2017	21.9 b	25.5 d	25.8 a	10.7 c	14.2 d	15.9 c	25.8 c	40.7 c	46.0 b
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
SF × Y	*P(F)*	*< 0.001*	*< 0.001*	*0.040*	*n.s.*	*0.011*	*< 0.001*	*n.s.*	*< 0.001*	*n.s.*

[a] The open-field experiment was carried out over 4 years in Carmagnola (Northern Italy) on three natural soils with different textures placed side by side; SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

[b] The starter fertilization results are based on 16 replicates (4 years × 4 repetitions). Control: no NP fertilization at planting; N: applied 27 kg N ha^{-1} as ammonium nitrate at planting; P: applied 69 kg P_2O_5 ha^{-1} as triple superphosphate at planting; NP: applied 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at planting.

[c] The year results are based on 16 replicates (4 starter fertilization treatments × 4 repetitions).

Means followed by different letters are significantly different. The level of significance (*P*(F)) is shown in the Table.

3.4.3. Growth stage development

The difference between the starter fertilization treatments recorded in the vegetative stages remained significant (*P* <0.001) at flowering and harvesting time (Table 4).

Table 4. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on the maize crop cycle, expressed as the date of flowering and grain moisture at harvest, for the field experiment carried out on soils with different textures in the 2014-2017 period.

Factor	**Source of variation**	**Date of flowering (DAS)**			**Grain moisture (%)**		
		SSL	**SL**	**SCL**	**SSL**	**SL**	**SCL**
Starter fertilization (SF)	control	97.3 a	95.9 a	96.5 a	28.3 a	26.3 a	28.5 a
	N	97.5 a	94.7 b	95.3 b	28.3 a	25.9 a	27.2 b
	P	96.4 b	94.9 b	96.6 a	28.1 a	26.0 a	28.1 a
	NP	94.9 c	92.4 c	93.4 c	26.1 b	24.0 b	26.3 c
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Year (Y)	2014	102.2 a	99.3 a	103.2 a	27.5 b	26.5 a	29.4 a
	2015	85.7 d	84.8 d	85.5 d	29.3 a	26.9 a	28.8 a
	2016	99.3 b	97.4 b	97.9 b	27.9 b	23.2 c	25.5 c
	2017	98.3 c	96.5 c	95.a c	26.4 c	25.5 b	26.4 b
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
SF × Y	*P(F)*	*n.s.*	*< 0.001*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Means followed by different letters are significantly different. The level of significance (*P*(F)) is shown in the Table. SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

The NP starter fertilization reduced the days to flowering by 2.4, 3.4 and 3.1 days and reduced the moisture content at harvest by 2.2%, 2.3% and 2.2%, in the SSL, SL and SCL soils, respectively, compared to the controls. On average, a significantly earlier flowering date (-0.9 days) was observed for the P fertilization in the SSL and SL soils and for the N fertilization in the SL (-1.1 days) and SCL (-1.3 days) soils. The single element fertilization led to a significant reduction of the moisture content in the grain at harvest, albeit only for the N fertilization in the SCL soil. The reduction in moisture content at harvest was significantly correlated to the reduction in days to anthesis (in all the years and soils the Pearson coefficients of correlation were between 0.64 and 0.93).

Only in the SL soil did a significant interaction occur for the flowering date: the advancement of the flowering date obtained with the NP fertilization was greater in 2016 (5.6 days) than in the other years (2.8 days). The interaction between the starter fertilization and year was never significant within each soil for the grain moisture at harvest.

3.4.4. Grain yield and components

A significant effect (*P* <0.05) of the starter fertilization on grain yield and on the yield components was observed in each soil (Table 5). The combined NP banded application led to a significant increase in grain yield in the SSL (+7%), SL (+15%) and SCL (+11%) soils. This starter fertilization treatment increased KSQ by 10% in all the soils and resulted in a higher number of kernels per ear. Moreover, the higher grain yields recorded for the NP starter fertilization in SL and SCL than in the controls is linked to significant increases in TKW of 5% and 3%, respectively. Only in the SL soil did the banded application close to the seed furrow of the single elements lead to a significant difference from the controls: the N fertilization increased grain yield by 9% and TKW by 3%. The interaction between the starter fertilization and year was never significant within any soil for the grain yield and TKW, while only in the SSL soil was a significant interaction observed for KSQ.

Table 5. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on the maize grain yield and the yield component, kernels per square meter (KSM) and thousand kernels weight (TKW), for a field experiment carried out on soils with different textures in the 2014-2017 period.

Factor	**Source of variation**	**Grain yield (t ha^{-1})**			**KSQ (n m^{-2})**			**TKW (g)**		
		SSL	**SL**	**SCL**	**SSL**	**SL**	**SCL**	**SSL**	**SL**	**SCL**
Starter fertilization (SF)	control	12.9 b	11.1 c	13.5 b	3721 c	3283 b	3789 b	355 b	341 b	361 b
	N	13.3 ab	12.1 ab	13.9 b	3880 bc	3447 ab	3869 ab	364 a	351 a	363 ab
	P	13.4 ab	11.6 bc	13.8 b	4034 ab	3490 ab	4012 ab	353 b	340 b	359 b
	NP	13.8 a	12.7 a	15.0 a	4142 a	3606 a	4123 a	351 b	357 a	371 a
	P(F)	*0.029*	*< 0.001*	*< 0.001*	*< 0.001*	*0.011*	*0.020*	*< 0.001*	*0.002*	*0.020*
Year (Y)	2014	12.8 c	11.9 bc	12.5 c	4091 a	3756 a	4277 a	365 a	350 b	356 b
	2015	12.1 c	10.6 c	14.3 b	3921 a	3748 a	4419 a	315 c	312 c	342 c
	2016	15.1 a	12.7 a	13.9 b	3714 b	3396 b	3468 b	369 a	371 a	380 a
	2017	13.7 b	12.4 ab	15.2 a	4078 b	2899 c	3663 b	364 a	355 b	375 a
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
SF × Y	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*0.016*	*0.056*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Means followed by different letters are significantly different. The level of significance (*P*(F)) is shown in the Table. SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

3.4.5. Qualitative traits

The starter fertilization significantly affected (P <0.05) the grain qualitative parameters at harvest (Table 6). Both the NP and N fertilizations alone led to a significant increase in grain hardness, expressed as both TW and energy required to grind the kernels (TME), in the SL and SCL soils. Conversely, no significant effect was observed for the P banded application alone, in any of the considered soils. On average, NP fertilization increased TW by 1 kg hl^{-1}, compared to the controls, in the SL and SCL soils, where the greatest advance in flowering date was recorded. NP fertilization significantly differed from the controls for GPC in all the soils: only in SSL for N alone did the starter fertilization lead to a higher protein in the kernel than the other treatments. The interaction between starter fertilization and year was never significant for any soil.

Table 6. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on maize quality, expressed as test weight (TW), grain hardness expressed as the total milling energy (TME) and grain protein content (GPC), for a field experiment carried out on soils with different textures in the 2014-2017 period.

Factor	**Source of variation**	**TW (kg hl^{-1})**			**GPC (%)**			**TME (J)**		
		SSL	**SL**	**SCL**	**SSL**	**SL**	**SCL**	**SSL**	**SL**	**SCL**
Starter fertilization (SF)	control	80.0 a	81.4 c	80.6 b	9.1 b	9.3 ab	9.0 a	1247 a	1277 b	1266 b
	N	80.1 a	81.9 b	81.2 a	9.4 a	9.5 a	9.0 a	1298 a	1331 a	1334 a
	P	79.9 a	81.5 bc	81.1 a	8.8 c	9.0 b	9.0 a	1265 a	1291 ab	1289 ab
	NP	80.6 a	82.4 a	81.5 a	8.9 bc	9.4 a	9.2 a	1228 a	1339 a	1326 a
	P(F)	*n.s.*	*< 0.001*	*0.001*	*< 0.001*	*0.004*	*n.s.*	*n.s.*	*0.021*	*0.004*
Year (Y)	2014	78.3 c	81.5 bc	80.8 bc	9.4 a	8.6 c	8.8 b	1227 b	1298 c	1278 b
	2015	79.5 b	80.3 c	80.4 c	8.3 c	9.4 b	8.7 b	1205 b	1350 b	1276 b
	2016	81.3 a	83.8 a	82.0 a	9.1 b	9.5 ab	9.2 a	1371 a	1407 a	1384 a
	2017	81.4 a	81.6 b	81.2 b	9.3 a	9.7 a	9.5 a	1229 b	1172 d	1277 b
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
SF × Y	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Means followed by different letters are significantly different. The level of significance (*P*(F)) is shown in the Table. SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

3.4.6. Sanitary traits

A significant effect ($P < 0.05$) of the starter fertilization was observed on the severity of ECB and fungal ear rot, and on FB contamination (Table 7 and Table 8).

The reduction in days to anthesis obtained as a result of the NP starter fertilization significantly reduced injuries caused by ECB in the SSL (-35%), SL (-55%) and SCL (-41%) soils. With the exception of the 2016 growing season, which was characterized by the lowest insect incidence, the ECB severity was significantly correlated to the reduction in days to anthesis (the Pearson coefficients of correlation ranged from 0.47 to 0.67).

The reduction of ECB, combined with the earlier ripening, as demonstrated by the grain moisture content at harvest, led to a lower ear rot severity (which was significant in the SSL and SL soils) and lower FB content (-74% and -70% for the SL and SCL soils, respectively). Fertilization banded close to the seeds with N or P alone did not lead to any significant difference from the untreated controls.

No significant effect of starter fertilization was observed for DON contamination.

As far as the severity of injuries on the maize ears and mycotoxin contamination are concerned, the interaction between the starter fertilization and year was never significant for any soil.

No significant correlations were found between ECB severity, grain yield, yield components or grain moisture content at harvest and ECB severity or fungal ear rot.

Table 7. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on European Corn Borer (ECB) and ear rot severity on maize ears at harvest for a field experiment carried out on soils with different textures in the 2014-2017 period.

Factor	Source of variation	ECB severity (%)			Ear rot severity (%)		
		SSL	SL	SCL	SSL	SL	SCL
Starter fertilization (SF)	control	3.7 a	5.4 a	3.7 a	7.0 a	5.7 a	4.0 a
	N	2.9 ab	4.4 ab	2.5 ab	5.8 ab	5.5 a	3.6 a
	P	3.7 a	3.8 bc	2.9 ab	7.4 a	3.9 ab	3.5 a
	NP	2.4 b	2.4 c	2.2 b	4.7 b	2.7 b	2.8 a
	P(F)	*0.026*	*0.001*	*0.025*	*0.003*	*0.001*	*n.s.*
Year (Y)	2014	4.3 a	4.9 a	4.4 a	14.8 a	7 a	5.5 a
	2015	4.7 a	5.9 a	3.6 ab	2.9 c	3.1 b	2.4 b
	2016	1.6 b	1.8 c	0.7 c	2.3 c	2 b	1.5 b
	2017	2.4 b	3.4 b	2.6 b	4.9 b	5.7 a	4.7 a
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
SF × Y	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Means followed by different letters are significantly different. The level of significance (*P*(F)) is shown in the Table. SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

Table 8. Effect of the starter fertilization with nitrogen (N), phosphorus (P) and their combination (NP) on fumonisins B1 + B2 (FBs) and deoxynivalenol (DON) contamination, for a field experiment carried out on soils with different textures in the 2014-2017 period.

Factor	**Source of variation**	**FBs (μg kg^{-1})**			**DON (μg kg^{-1})**		
		SSL	**SL**	**SCL**	**SSL**	**SL**	**SCL**
Starter fertilization (SF)	control	1136 a	1607 a	2211 a	11933 a	2679 a	3499 a
	N	839 a	1184 ab	1298 ab	10953 a	2863 a	2710 a
	P	866 a	1095 ab	1249 ab	11380 a	3529 a	3464 a
	NP	671 a	415 b	658 b	8032 a	2160 a	2016 a
	P(F)	*n.s.*	*0.022*	*0.016*	*n.s.*	*n.s.*	*n.s.*
Year (Y)	2014	1021 a	381 b	883 b	38303 a	11715 a	10615 a
	2015	1103 a	2455 a	1418 b	231 b	93 b	981 b
	2016	565 a	338 b	637 b	1086 b	165 b	415 b
	2017	830 a	1491 a	2566 a	789 b	211 b	660 b
	P(F)	*0.414*	*< 0.001*	*0.016*	*< 0.001*	*< 0.001*	*< 0.001*
SF × Y	*P(F)*	*n.s.*	*n.s.*	*0.046*	*n.s.*	*n.s.*	*n.s.*

Means followed by different letters are significantly different. The level of significance (*P*(F)) is shown in the Table. SSL: sandy silt loam, SL: silt loam, SCL: silty clay loam, see Table 1 for details on the soil properties.

3.5. Discussion

This study provides useful information on how starter fertilization strategies with sub-surface banded placement at sowing affect early maize development, and consequently the yield components and the qualitative and sanitary grain traits. Data collected from field experiments, carried out on three types of soil, placed side by side, and over four growing seasons, highlight that the starter fertilization with P or N in bands close to maize seed furrows always led to benefits in terms of a faster early plant growth. A higher early plant vigor, e.g. plant height at the leaf emergence and stem elongation stages, or quantified through the NDVI vegetative index, was observed as a result of the application of a single nutrient, although the effects differed according to the soil properties. The starter P fertilization led to positive effects on early plant vigor and shortened time to flowering in both the SSL and SL soils, which were characterized by the lowest and highest P availability, respectively. Bermudez and Mallarino, (2002) reported that the magnitude of increased plant growth due to starter fertilization increased when the starter was applied to low phosphorus soil, although the growth response in their experiment was also significant when the P content of the soil was high, and could therefore also lead to grain yield increases (Quinn et al., 2020). Roth at al. (2006) also reported that the early season effects of a P-based starter fertilizer were also possible for soils that test high in P.

Early plant vigor benefits of the N only starter fertilization were recorded for all the soil types studied. N, although applied at a lower rate, on average favored a better initial growth of maize than P alone in finer textured soils (SL and SCL).

These findings suggest that the localized application of N close to seed furrows increases the use efficiency of this nutrient, thus resulting in a higher crop nutrition, which in turn is able to enhance early biomass accumulation and grain yield (Niehuses et al., 2004). Nkebiwe et al. (2016) observed that N fertilizers alone overall resulted in a higher yield benefit than sub-surface banded P application. Ma et al. (2015) reported a higher shoot dry weight of maize in the early growth stages with the sub-surface banded distribution at sowing of 46 kg

N ha^{-1}, compared to the same application of 32 kg P_2O_5 ha^{-1}, thus confirming the overall trend observed in our experiment, even though P was applied at sowing at a 2.5 higher rate than N.

Although the positioning of N or P close to the seed furrows led to a positive effect on early plant development, these benefits, quantified by means of the AUCDC index, were influenced to a great extent by the soil properties, and by the meteorological trends during the early vegetative stage. Single nutrient starter applications of N or P both reduced the risk of a delay in maize development in years with cooler soil temperatures, in part as a consequence of a higher incidence of rain during spring. Only in 2014, which had a lower precipitation frequency in the first weeks after sowing, were no difference in plant development detected after the N or P starter fertilizations. This information underlines that the physical properties of the soil, and in particular its ability to warm up more quickly in spring, despite frequent rainfall events, play an important role in the early development of maize, which is equally important for the soil N and P fertility.

Furthermore, although the positioning of single P or N close to plants at sowing resulted in more rapid early crop development, there were only a few instances in the 4-year experimental period, where this positive effect in crop early vigor was able to lead to productive and qualitative benefits in terms of grain yield. In contrast, when P and N were applied together in bands close to seed furrows, the maize vigor in the early stages was always significantly higher than a single element application. The improvement in shoot growth in response to an NP starter fertilization is primarily a consequence of the development of a deeper and more extensive root system, as a result of the synergistic response to the supply of critical nutrients (Ma et al., 2013).

A faster root system establishment, stimulated by NP fertilization, also leads to a more effective use of other nutrients, such as potassium, by the crop (Mallarino et al., 2011) and in general plays a crucial role in the overall nutrition of a plant. Nkebiwe et al. (2016) found, in a meta-analysis based on several crops, that the placement of a combination of ammonium and soluble P was more effective than an application of these fertilizers alone, suggesting a synergistic effect with a stronger root signalling and root-growth, in particular as far as the growth of lateral fine roots was concerned (Jing et al., 2010). Ma et al. (2015) and Jing et al. (2012) reported that the form of applied N can affect both the N and P uptake of plants. In our experiment, N was supplied in an ammonium (N-NH_4^+) form. A previous study, carried out on calcareous soils with a low P status, showed that supplying N as N-NH_4^+ can increase the availability and uptake of P in a soil, following rhizosphere acidification during the acquisition of N by maize due to the release of protons from the roots (Jing et al., 2010). The occurrence of NH_4-induced rhizosphere acidification could also lead to a better initial nutrition of microelements such as Fe, Zn and Mn (Ma et al., 2014). Moreover, Jing et al. (2012) reported that the root density and extension were greater when the N source was ammonium rather than nitrate or urea, and this led to an overall improved spatial nutrient acquisition and chemical mobilization.

As a consequence of the better early root growth and functionality, an early establishment of a larger leaf area allowed a progressively greater radiation interception to be achieved, which increased over the course of the growing season and resulted in greater biomass accumulation and radiation interception throughout the vegetative stages. The more rapid increase in NDVI in our experiment was consistent with this. In this context, the presence of N in the starter fertilization could directly enhance photosynthesis and leaf area development, thus offering another possible explanation for the positive interaction of the localized application of N and P (Jing et al., 2010).

In all the soils and for all the years, the NP starter fertilization significantly reduced the delay in plant development and favoured a significantly earlier flowering, compared to the fertilization at sowing with single elements. Thus, the NP-based fertilizer always resulted in a significant increase in grain yield and an earlier ripening, as quantified by the lower grain moisture content at harvest. These data suggest yield increases were closely correlated with early flowering, and this has formed the basis of such agronomic strategies as early maize sowing, to the extent this is possible in temperate environments. Early flowering, with the maximum LAI and the highest physiological activity of the leaves, leads to a better exploitation of the available radiation, which is at its peak at around the summer solstice. Moreover, since the GDD accumulated during the flowering of the flowering period are higher than those of during the final ripening phase, an advance in the flowering date by a few days could be translated into an advance of at least 7-12 days in the dry-down process and in the harvest date. Thus, the adoption of agronomic practices that are able to reduce the delays that occur in vegetative development as a consequence of rain and temperature drops in the early development stage can maximize the benefits of a timely sowing.

In order to translate the early vigor of maize into grain yield increases, it is necessary to reduce the interval between sowing and silking: on average, in the present experiment, NP starter fertilization advanced flowering by 2.4 to 3.1 days, while the sub-surface P- or N-banded fertilization allowed a maximum improvement of 1 day in the most responsive conditions. Kaiser et al. (2016) reported that in-furrow NP fertilization, at a lower rate than our sub-surface banded application, shortened the period between sowing and silking by approximately 1 day, but resulted in no difference in the grain yield. In our experiment, the yield benefits from NP starter fertilization were between 7% to 15%, according to the type of soil, thus confirming the finding of Quinn et al. (2020), who demonstrated a grain increase of +9%, regardless of the sub-surface placement across a combination of various environments and management practices in the U.S.

The yield gain was higher in soils under cooler conditions as a consequence of the finer textures and the slower water drainage, and also the enhanced N and P availability. The increase in yield was mainly related to the increase in the ear length and KSQ, while the higher TKW played a secondary role, in agreement with the results of Tsimba et al. (2013). A P deficiency generally decreases the number of seeds produced more than any reductions in the seed size (Grant et al., 2001). Both of these yield components were positively influenced by a higher proportional radiation interception during the plant crop cycle, although in different phenological phases. A reduction in the crop growth rate, as a consequence of a low radiation interception during flowering, was associated with a small number of grains, which is the main yield determinant (Andrade, 1995). Ma et al. (2015) reported a close relationship between grain yield and kernel number per ear with the plant growth rate during the critical 2-week period around silking and highlighted a positive effect of NP starter fertilization on these parameters.

In addition to yield benefits, the higher light interception and temperature during dough ripening permit kernels to be obtained with a higher TW and TME. It is well known that when maize ripening occurs in a warm year, the kernels are harder because of a more compact starch accumulation (Testa et al., 2015). Thus, an earlier flowering date could deliver a qualitative improvement to maize kernels destined for the food dry-milling supply chain, in which a high degree of hardness is requested (Blandino et al., 2013).

Moreover, sanitary traits, such as the mycotoxin content, could also be improved by the application of practices that are able to advance maize flowering. Although the DON content is closely related to a delay in the maize harvest (Blandino et al., 2009b), the reduction in the period between sowing and anthesis obtained with sub-surface banded fertilizers did not result in a significant reduction in the accumulation of these contaminants. However, the earlier flowering, that occurred in response to the use of an NP starter fertilization, was able to reduce the risk of fumonisin contamination. The relationship between insect ear injuries and these contaminants is well known in temperate growing areas (Blandino et al., 2009a). An early sowing date is a key practice in reducing ECB severity, since insect larva feeding occurs on harder and drier kernels, and molds caused by larvae could take less advantage of the colonization of a less compact substrate, as was observed for a late ripening period (Blandino et al., 2009c). Similarly, the effect of starter fertilization on the early vigor of maize resulted in a shift in the insect activities to a later ear development stage, thereby reducing fumonisin contamination. Moreover, since the harvest date in the present experiment was the same for all the compared treatments, the role of earlier flowering in minimizing fumonisin, and perhaps other mycotoxins, could be higher in later flowering crops with a more extended dry down period and later harvest. In fact, a lower moisture content at harvest can be translated into the possibility of harvesting earlier at the same kernel growth stage. In this way, the period when grains are present in the field is reduced as is the period in which the fungal species producing mycotoxins find the right conditions to accumulate these contaminants (Blandino et al., 2017a; 2009c).

As far as the control of insects is concerned, another benefit of a starter fertilization that has not been quantified in the present research is that of the lower damage caused by such soil insects as wireworms (*Agriotes* spp.) and Western Corn Rootworm when the early growth of maize is rapid (Blandino et al., 2017b).

3.6. Conclusions

In conclusion, the results of these field experiments, carried out with a timely sowing in an area where sub-optimal growth conditions could negatively affect the early vigor of maize, have highlighted the advantages of the adoption of NP starter fertilization on maize development, and consequently on both grain yield and quality. The synergistic effect of N and P and their consistent effect in promoting early flowering, justifies their combined application in sub-surface sowing bands in different production situations, even in soils that have tested high to P or N. This experiment has in particular pointed out that the agronomic and productivity benefits of applying an NP-based starter fertilizer could be even higher in fine-texture soil. Thus, NP starter fertilization is a crop practice that enhances the agronomic benefits of timely sowing in cooler production environments to a great extent, and this application needs to be evaluated in broader agronomic terms rather than simply considering it as a means of satisfying nutritional needs. Overall, starter fertilization seems to be a key strategy for the efficient management of N and P in temperate maize production systems. Furthermore, in order to guarantee an efficient use of sub-surface banded fertilization, particularly for those conditions where agro-environmental rules strictly regulate N and P application, it will be necessary to carefully evaluate the role of the starter fertilizer rate, in order to define profitable management solutions designed for the specific production conditions.

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4. Maize response to NP starter fertilization in mineral-fertilized or manured systems

Authors: Michela Battisti[1], Barbara Moretti[1], Massimo Blandino[1], Carlo Grignani[1], Laura Zavattaro[2*]

Affiliation:
[1] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy
[2] Department of Veterinary Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy

**Corresponding author:* Laura Zavattaro, Department of Veterinary Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy
E-mail: laura.zavattaro@unito.it

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4.1. Abstract

Phosphorus (P) is an essential nutrient for maize production, but in temperate areas the P uptake during early growing stages can be limited due to low soil temperature, even though the soil has tested high in P. The objective of this study was to assess the effects of starter nitrogen and phosphorous (NP) fertilisation during early growth stages and its carryover until maize harvest, in mineral-fertilised or manured systems. We hypothesised that P starter fertilization might be avoided in cropping systems characterised by positive accumulated P balance due to excessive P fertilisation over the past.

A field experiment was carried out in NW Italy during the 2019 and 2020 growing seasons. The trial compared sub-surface placement of NP (diammonium phosphate) or N alone (ammonium nitrate) in bands close to the maize seed furrows, in differing long-term (LT) fertilisation managements: two doses of urea (Min-L and Min-H), two doses of bovine slurry (Slu-L and Slu-H) or two doses of farmyard manure (Fym-L and Fym-H). The two rates, low (L) and high (H), corresponded to 170 and 250 kg N ha^{-1} year^{-1} respectively.

Compared to N fertilisation, NP starter fertilisation improved early maize growth assessed by leaf area index (LAI) and shoot dry weight (SDW) in all systems. The effects differed between the two years (2019: LAI +63%, SDW +67%; 2020: LAI +36%, SDW +38%), as 2019 was cool during the first growth. Higher LAI and SDW values were confirmed at crop flowering in the mineral-fertilised systems only. As shoot growth was enhanced by NP starter fertilisation, anthesis was anticipated by 1 day in all systems. However, a response to NP starter fertilisation at harvest was recorded in mineral-fertilised systems only (+1.3 and +3.2 t ha^{-1} in Min-L and Min-H, respectively). The uptake of P, used as a true indicator of soil nutrient availability, increased with increasing soil Olsen P until 39 mg kg^{-1}. These results suggest that soil test thresholds should be revised for points above which P fertilisation should be suspended.

Keywords: Long-term fertilisation, farmyard manure, bovine slurry, urea, diammonium phosphate

4.2. Introduction

Phosphorus (P) is an essential nutrient required for crop growth and production; therefore, it plays a fundamental role in soil fertility and world food security (Mitran et al., 2018; Scholz et al., 2013). Soils contain a large amount of P relative to plant requirements, but most forms of P in the soil have a very low solubility and low availability for plant uptake (Zhu et al., 2018; Kratz et al., 2019). For these reasons, mineral P fertilisers containing highly-soluble P are traditionally applied to maintain an optimal soil P status in intensively-managed agricultural systems (Richardson et al., 2009). However, phosphate rocks that are the major current source of P for fertilisers production are non-renewable, which puts the future P fertiliser supply at risk (Scholz and Wellmer, 2013).

Globally, maize (*Zea mays* L.) is the most produced cereal as it is used for animal feed, human consumption and industrial or energy uses. It is the main arable crop cultivated in the Po Plain (Northern Italy), where it is intensively cropped and used for livestock farming (Zavattaro et al., 2012; Fumagalli et al., 2011). Maize is also one of the most sensitive crops to P supply (Zicker et al., 2018). Indeed, limited P supply usually impairs maize growth, as it delays phyllochron, reduces leaf elongation and final leaf size, and limits aboveground biomass (Plénet et al., 2000a; Plénet et al., 2000b). Grant et al. (2001) also reported that an adequate P supply is important for early crop establishment and consequently for the final maize yield. In temperate areas, the P uptake of young maize plants can be limited as a consequence of cold stress, even though soils test high in P using standard extraction methods (e.g., Olsen). Reduced plant P utilisation in these circumstances could be due to different factors, such as little P soil mobility, decreased rates of plant P uptake, and limited root system development (Mollier and Pellerin, 1999; Imran et al., 2013). Low temperatures can also inhibit microbial activity, that plays a key role when P is supplied as organic sources, as they require a biological process to make nutrients available for the plant (e.g., extracellular enzymes release by soil microorganisms and plant roots for P solubilisation) (Jones and Oburger, 2010).

It is widely acknowledged that P fertilisation is an issue in European agriculture and politics. To date, there is no common regulation on the application of P fertilisers at the European level (Garske et al., 2020), even though P excess has been recognised in several regions, associated sometimes - but not always - with high livestock density (Tóth et al., 2014; Einarsson et al., 2020). In livestock farming systems, most farmers pay little attention to the P fertiliser value of manures (Hooda et al., 2001). Indeed, in many European countries, farmers commonly supply P through organic sources in excess of crop requirements, opting instead to manage the nutrient management plan on N fertiliser and/or on the restriction by the Nitrates Directive (91/676/EEC), without considering the disequilibrium between the N/P ratio in organic fertilisers and in crops (Amery and Schoumans, 2014). This surplus leads to P accumulation in soils and increased risk of P losses to water (Borda et al., 2011; Borda et al., 2014). Aside from the environmental problem, maize growers are keen to avoid early-stage P deficiency to minimize the risk of crop yield penalties, so they routinely apply a dose of mineral P combined with nitrogen (N) near the seeds at sowing even in manured soils, as a starter fertilisation (Schröder et al., 2015). The combination of N and P was proved as an effective stimulator for both lateral and fine root proliferation in several studies (e.g., Jing et al., 2010; Ma et al., 2013). Additionally, plant uptake of ammonium N lowers rhizosphere pH, which can locally increase P availability (Jing et al., 2012). In the context of intensive agriculture, it is crucially important to balance the opposing needs of reducing the potential risks of low yields while protecting the environment. Any decision on fertilisation needs to be done while considering soil type, soil P status, and weather conditions.

To this end, this work evaluated the effect of starter NP fertilisation at sowing, in six different fertilisation managements of continuous maize for grain cropping systems. The six systems included long-term (LT) fertilisation with mineral fertilisers or animal manures (i.e., bovine slurry and farmyard manure) that resulted in different initial soil P availabilities, as estimated by a standard soil test. The NP starter fertilisation at sowing was compared with a N only addition. Measurements were focused on the early growth phase, but extended until harvest to assess yield quantity and quality. We also tested the extent to which the LT fertilisation of a system influenced the benefits of starter fertilisation on the crop.

4.3. Materials and methods

4.3.1. Long-term fertilisation at Tetto-Frati

Measurements were carried out in 2019 and 2020 growing seasons on selected plots of the LT experiment of Tetto Frati (44°53’N; 7°41’E; 232 m a.s.l.) of the University of Turin (NW Italy).

The LTE, established in 1992, was described by Grignani et al. (2007), Zavattaro et al. (2012) and Zavattaro et al. (2016), among others. The LTE, a complete randomised block with three replicates on 75 m^2 plots, compared four cropping systems based on maize, at five N application levels. Here we analyse only data regarding the continuous maize for grain system at two doses of mineral fertiliser as urea (Min-L and Min-H), two doses of bovine slurry (Slu-L and Slu-H), and two doses of farmyard manure (Fym-L and Fym-H) as sources of N, as LT fertilisation. Each fertiliser was supplied at two rates, low (L) and high (H), corresponding to 170 and 250 kg N ha^{-1} $year^{-1}$ respectively, since 2011. The Min and Slu systems also received a supplement of 180 kg K_2O ha^{-1} as KCl. The plots had also received different amounts of mineral P fertilisers along the years, thus leading to marked differences in the soil P content, as detailed hereafter. The soil background P availability was here used to study the interaction of starter P fertilisation with three different LT strategies.

The soil texture is loam and the soil is classified as Typic Udifluvent (Soil Survey Staff, 2014). The ploughed layer (0–30 cm) contains 48.2, 44.3 and 7.5% of sand, silt and clay, respectively, and has a sub-alkaline soil pH (8.1 measured in water at 1:2.5 *w/v*). The Cation Exchange Capacity is also low (10.1 $cmol_{(+)}$ kg^{-1}), and so is the exchangeable K (14.4 mg kg^{-1}). Soil Olsen P, soil organic carbon (SOC) and total N (N_{tot}) concentrations measured at each treatment in March 2019, at the start of the experiment here described, are reported in Table 1. The highest soil Olsen P concentration was recorded in Fym-H (91 mg kg^{-1}), followed by Fym-L (52 mg kg^{-1}). On the contrary, Min treatments did not show differences between fertiliser rates and had the lowest values, while both Slu-L and Slu-H were intermediate (c. 29 mg kg^{-1} of Olsen P). As expected, the SOC concentration was highest in the Fym-H treatment (1.13%), and the lowest in either Min-H, Min-L (0.72%) and Slu-L (0.81%). Similarly, the highest N_{tot} concentration was found in the Fym-H treatment (1.33%), and the lowest in either Min-H or Min-L (0.86 and 0.85%). The C/N was c. 8.0-8.8 and did not differ between LT fertilisation strategies.

Table 1. Soil Olsen P, organic carbon (C) and total nitrogen (N) concentrations of each LT fertilisation at the start of the experiment in 2019. Means followed by different letters are significantly different for each parameter.

	Olsen P (mg kg^{-1})	**Organic C (%)**	**Total N (g kg^{-1})**
Min-L	12.7 c	0.72 b	0.86 b
Min-H	15.1 c	0.72 b	0.85 b
Slu-L	29.4 bc	0.81 b	1.01 ab
Slu-H	29.8 bc	0.91 ab	1.10 ab
Fym-L	51.9 b	0.91 ab	1.04 ab
Fym-H	91.4 a	1.13 a	1.33 a
P(F)	*< 0.001*	*0.003*	*0.008*

The climate at the site is temperate sub-continental, with by two main rainy periods in spring and autumn. Daily temperature and precipitation were measured at a meteorological station located in the experimental platform. The accumulated growing degree days (GDDs) for maize were calculated considering 10°C as the minimum base temperature and 30°C as the maximum temperature threshold.

4.3.2. Experimental fertilisation and agronomic management

The six LT fertilisation plots (Min-L, Min-H, Slu-L, Slu-H, Fym-L and Fym-H) were split into two emiplots (30 m2, corresponding to four maize rows) to set two different management options for P fertilisation. At maize sowing, one emiplot received the banded application of 27 kg ha^{-1} of N and 69 kg ha^{-1} of P_2O_5 as diammonium phosphate (DAP, 18% N and 46% P_2O_5 w/w; hereafter indicated as NP), while the second emiplot received 27 kg N ha^{-1} as ammonium nitrate (34% N w/w; hereafter indicated as N). Diammonium phosphate and ammonium nitrate were deposited 5 cm apart from the seed furrows and at a depth of 10 cm, using a calibrated granular dispenser applied to the planter (Monosem NG, Largeasse, France). No further mineral P fertiliser was distributed in the two experimental years. The crop was managed similarly in all plots. Soil was hoed in autumn and maize residue (stalks, cobs and bracts) were incorporated. All fertilisers, both mineral and organic, were surface supplied in spring and immediately incorporated with disk harrowing. The chemical properties of the bovine slurry and farmyard manure used for the field experiment are reported in Table S1. Slurry supplied 8 and 12 kg P ha^{-1} in 2019, or 30 and 44 kg P ha^{-1} in 2020, in Slu-L and Slu-H treatments, respectively, while farmyard manure supplied 21 and 32 kg P ha^{-1} in 2019, or 28 and 41 kg P ha^{-1} in 2020, in Low and High rate treatments, respectively.

The mechanical maize seeding was carried out on 1st April 2019 and 3rd April 2020, using the Corteva Agriscience™ P1547 hybrid (FAO maturity class 600, 130 days relative to maturity). The distance between the plants and the plant rows were 0.16 and 0.75 m, providing a crop density of 8.3 plant m^{-2}. Plots were weeded in pre- and post-emergence. Sprinkler irrigation supplied c. 40 mm per year.

4.3.3. Crop development and nutrient uptakes

In order to assess early crop development, different measurements were made on the two central rows of each emiplot, over a length of 5 m. Some measurements were planned based on days after sowing (DAS) as the time scale, while others were expressed as related to the plant growth stage (GS), according to the BBCH scale. A hand-held optical sensing device, GreenSeekerTM® (Trimble©, Sunnyvale, California, the USA), was used to measure the Normalized Difference Vegetation Index (NDVI) during vegetative stages. The measurement, made holding the instrument c. 60 cm above the plant canopy, was performed approximately every 7 days starting from the two-leaf stage (GS12) until tassel emission (GS53). The Area Under Canopy Development Curve (AUCDC) was calculated for each treatment using NDVI measurements at each observation date, following the formula (1) proposed by Capo et al. (2020):

$$AUCDC = \sum_{i}^{n-1} \{[(R_i + R_{i+1})/2]\,(t_{i+1} - t_i)\} \tag{1}$$

where R is the NDVI value, t is the time of observation and n is the number of observations.

The maize plant height was monitored during the vegetative stages by measuring five randomly chosen plants from the ground level up to the collar of tallest fully developed leaf (GS13 - GS19) or up to the tallest detectable node (GS30 - GS53). Plant height was measured five times from 45 to 74 DAS. Plant height was linearly interpolated with time expressed as DAS (Table 3, Fig. S1) to derive the growth rate expressed as cm d^{-1} for each measurement interval, separately at each treatment.

Three plants per plot were sampled at two stages, 50 DAS and flowering, to determine shoot dry weight (SDW), leaf area index (LAI) and tissue P and N concentrations. LAI was measured using a planimeter (Delta-T Devices Ltd, Cambridge, UK). The plant total P content was quantified after mineralisation in a muffle furnace at 450 °C for 5 h with spectroscopy under continuous-flow conditions (Evolution II, Alliance), while total N was assessed with a CN elemental analyser (Flash EA 1112, Thermoquest, MIPAF method, 2000, Italy), using atropine ($C_{17}H_{23}NO_3$, Merk Analytical) as the analytical standard and ERM-BC381 rye flour as the reference material.

The plant flowering date, expressed as DAS, was determined when >50% of the plants had the tips of stigmata visible (GS63).

4.3.4. Grain yield and sanitary traits

Maize was manually harvested from areas of 7.5 m^2 from each subplot at maturity, to quantify the grain yield, grain quality and biomass production.

Harvest took place on 19th September 2019 and on 16th September 2020. Measurements also included the number of plants and fully developed ears per surface unit. The number of kernels per square meter (KSQ) was calculated multiplying the number of kernels per ear (determined on 7 randomly selected ears) by the number of ears per square meter, as reported by Testa et al. (2016). Sub-samples of 12 ears were shelled using an electric single-ear sheller. Grain moisture was determined using a Dickey-John GAC100 grain analyser (Auburn, IL, USA). Grain, cob and stover were oven dried at 60°C for 72 hours and weighed separately. A sub-sample of 200 kernels was weighed to obtain the thousand kernels weight (TKW).

The grain protein content was obtained by multiplying N grain content (assessed as described above for the tissue N concentration) by a standard 6.25 coefficient. The sanitary traits were evaluated by the fumonisin B_1 and B_2 (FBs) contamination using the ELISA method, by means of direct competitive immunoassays (RIDASCREEN® Fumonisin, R-Biopharm, Darmstadt, Germany), according to the manufacturer's instructions.

4.3.5. Soil N and P measurements

The soil was sampled at 50 DAS, at 75 DAS and at flowering. Three 0-30 cm deep soil cores were collected with an auger along the central rows of each plot and pooled together to obtain a representative sample. The soil mineral N was extracted with 1 M KCl solution shaken for 1 hour with 70 g of wet soil, then filtered and determined by colorimetry with a continuous flow analyser (Evolution II, Alliance Analytical Inc., Menlo Park, CA). Part of the soil sample was air-dried and sieved through a 2-mm mesh screen to analyse the plant-available P using the Olsen method (Olsen et al., 1954).

A linear-plateau model was used to interpolate soil Olsen P *vs* the true soil available P for the crop, as assessed by the plant uptake (2):

$$Plant\ P\ uptake = \begin{cases} a * (Olsen\ P) + b, & Olsen\ P < C \\ k, & Olsen\ P \geq C \end{cases} \tag{2}$$

where *a* and *b* are shape coefficients, *k* is the predicted total P uptake plateau, and *C* is the critical value of soil Olsen P (mg P kg^{-1}) after which plant uptake is not influenced by soil availability. The non-linear least square (n.l.s.) function in the R software statistical package was used as fitting procedure.

During 2019, and for the high rate of each LT fertilisation only, six enzymatic activities involved in key steps of P cycle were measured: acid (acP) and alkaline phosphomonoesterase (alkP), phosphodiesterase (bisP), pyrophosphodiesterase (piroP), inositol-P phosphatase (inositP) and nonanoate esterase (nona) involved in the hydrolysis of ester bonds. Enzymatic activities were measured in duplicate at each of the three field replicates, in the 0-30 cm soil layer sampled at 50 DAS. Enzymes were desorbed as described Cowie et al. (2013) using a heteromolecular exchange method via bead-beating in order to disrupt microbial cells and soil aggregates.

4.3.6. Statistical analysis

The experiment was analysed as a split-plot design, where the LT fertilisation (i.e., Min-L, Min-H, Slu-L, Slu-H, Fym-L and Fym-H), is the main factor, while the starter fertilisation adopted in each emiplot (N or NP) is the sub-factor. A mixed effects model was used, where LT fertilisation, starter fertilisation and year were considered as fixed factors, while block, plot and emiplot (as nested effects), as well as the interaction between block and plot with the year, were considered as random factors. A graphical method was used to verify the basic assumptions (Onofri et al., 2016). When single factors or their interaction determined a significant effect, means were compared using the Bonferroni post hoc test at the $P \leq 0.05$. The lme function in the nlme statistical package of the R software (R Core Team, 2019) was used for analyses.

4.4. Results

4.4.1. Weather conditions and soil temperature

The two experimental years showed slight differences in the meteorological trends for both temperatures and rainfall during maize growing seasons (Fig. 1). During the first maize growing phase - April and May - the air temperature was lower in 2019 than in 2020, leading to reduced accumulated GDDs (281°C d^{-1} in 2019, and 394°C d^{-1} in 2020, as a sum of April and May). During the second maize growth phase, from stem elongation to flowering, corresponding to June and July, the accumulated GDDs were higher in 2019 (756°C d^{-1} in June and July), than in 2020 (690°C d^{-1}). Finally, August and September, corresponding to ripening stages, were similar according to the GDD indicator in the two experimental years (~670°C d^{-1} in August and September).

The rainfall amount showed marked differences over the two years in May (+21 mm in 2020), June (+104 mm in 2020), July (-82 mm in 2020) and September (-63 mm in 2020).

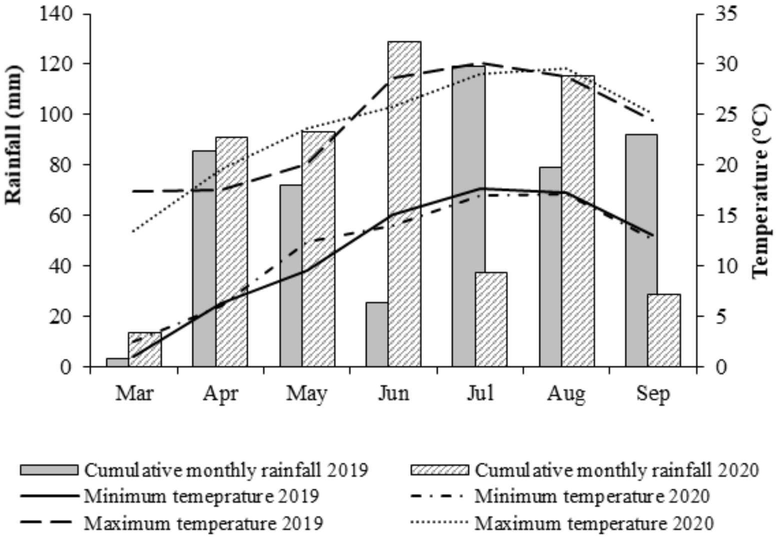

Figure 1. Rainfall and average minimum and maximum air temperature over the 2019 and 2020 growing seasons. All records are from the weather station at the Tetto Frati experimental site.

4.4.2. Leaf area index and shoot biomass production

The starter fertilisation influenced early maize production in the three LT fertilisation systems. Generally, leaf area and shoot dry weight (SDW) were significantly higher in the treatment with the sub-surface band application of NP compared to that with N only (Table 2).

Table 2. Effect of long-term (LT) fertilisation, starter fertilisation and year on the leaf area index (LAI) and shoot dry weight (SDW) production at 50 days after sowing (DAS) and flowering (FL) stages. Means followed by different letters are significantly different (Bonferroni post hoc test) for each parameter. The level of significance, P(F), is shown in the table, n.s.: non-significant.

	Source of variation	**LAI (m^2 m^{-2})**		**SDW (g m^{-2})**	
		50 DAS	**FL**	**50 DAS**	**FL**
LT fertilization	Min-L	0.6 b	5.2	37.3 b	1290 b
	Min-H	0.5 b	5.4	30.8 b	1252 b
	Slu-L	0.8 a	5.3	59.2 a	1396 ab
	Slu-H	0.9 a	5.7	64.1 a	1401 ab
	Fym-L	0.9 a	5.4	62.8 a	1385 ab
	Fym-H	1.0 a	5.7	69.0 a	1539 a
	P(F)	*< 0.001*	*n.s.*	*< 0.001*	*0.021*
Starter fertilization	N	0.6 b	5.1 b	44.5 b	1236 b
	NP	0.9 a	5.8 a	63.2 a	1518 a
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Year	2019	0.4 b	5.6	14.8 b	1279 b
	2020	1.1 a	5.3	93.0 a	1475 a
	P(F)	*< 0.001*	*n.s.*	*< 0.001*	*< 0.001*
LT × Starter	*P(F)*	*n.s.*	*0.003*	*0.021*	*< 0.001*
LT × Year	*P(F)*	*< 0.001*	*n.s.*	*< 0.001*	*n.s.*
Starter × Year	*P(F)*	*< 0.001*	*n.s.*	*< 0.001*	*n.s.*
LT × St × Year	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

However, the growing pattern was influenced by external temperature, as indicated by a significant interaction starter fertilisation × year at 50 DAS (data not shown), roughly corresponding to the first growing phase (April and May). Indeed, NP increased the LAI by 63% compared to N in 2019, and only 36% in

2020. Similarly, the SDW was enhanced by NP fertilisation in the year with a cool first phase, 2019, (+67%) compared to 2020 (+38%).

The interaction LT fertilisation × starter fertilisation, that indicates a different impact of starter P supply over the three LT fertilisation patterns, was significant both at 50 DAS and flowering for SDW, and only at flowering for LAI (Fig. 2a, b, c and d). The NP starter fertilisation increased SDW at 50 DAS by 2.1 and 2.3-fold in Min-L and Min-H treatments, and by 1.3 and 1.6-fold in Slu-L and Slu-H treatments, while the increase was 1.1-fold in both Fym treatments. At flowering, LAI was 31 and 35% greater in NP than in N treatments in Min-L and Min-H systems, respectively, and so was the shoot dry weight (+1.7- and +1.6-fold greater than in the N treatment, respectively). Furthermore, the NP starter fertilisation caused an increase in shoot biomass by 17% in Fym-H, despite no significant differences were found in LAI.

4.4.3. Crop early vigor, flowering date and grain moisture content

The starter fertilisation with NP enhanced early crop development if compared with N only, when applied to Min systems, but rarely when associated to LT organic fertilisation (Table 3; Fig. 2e). The year also influenced the plant early growth response to starter fertilisation. This was shown both by growing rate and AUCDC indicators. The growing rate increase due to NP compared with N recorded in Min systems was more pronounced in 2019 (+37 and +26% for Min-L and Min-H, respectively) compared to 2020 (+11 and +21%), while within LT organic fertilisations only the Slu-H treatment showed an increased growing rate (+12%) after NP starter fertilisation, during the first year of experiment (data not shown). Similarly, the AUCDC index, that condenses differences in NDVI measurements over time (Fig. S2), showed a significant LT fertilisation × starter fertilisation interaction (Table 3). The starter fertilisation affected plant development during vegetative stages until flowering in both Min systems and Slu-H, in both growing seasons (Fig. 2f). The AUCDC index pointed out that NP increased by 18, 23 and 6% the early crop development of Min-L, Min-H and Slu-H treatments, respectively.

Table 3. Effect of LT fertilisation, starter fertilisation and year on the growing rate, area under the canopy development curve (AUCDC), date of flowering (expressed ad days after sowing, DAS) and grain moisture content. Means followed by different letters are significantly different (Bonferroni post hoc test) for each parameter. The level of significance, P(F), is shown in the table, n.s.: non-significant.

	Source of variation	**Growing rate (cm d^{-1})**	**AUCDC**	**Flowering date (DAS)**	**Grain moisture content (%)**
LT fertilization	Min-L	4.3 b	22.8 c	92.1 a	23.5 b
	Min-H	3.9 b	21.0 c	93.4 a	24.6 a
	Slu-L	4.9 a	26.0 b	90.1 b	22.5 c
	Slu-H	5.0 a	27.0 ab	89.3 b	23.7 ab
	Fym-L	4.9 a	27.4 ab	89.2 b	22.9 bc
	Fym-H	5.2 a	28.2 a	88.8 b	23.6 b
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Starter fertilization	N	4.5 b	24.4 b	91.0 a	23.7 a
	NP	4.9 a	26.4 a	90.0 b	23.3 b
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*0.025*
Year	2019	2.6 b	21.2 b	96.7 a	25.0 a
	2020	6.8 a	29.6 a	85.3 b	21.9 b
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
LT × Starter	*P(F)*	*0.025*	*0.004*	*n.s.*	*n.s.*
LT × Year	*P(F)*	*n.s.*	*< 0.001*	*n.s.*	*0.001*
Starter × Year	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*0.018*
LT × St × Year	*P(F)*	*0.029*	*n.s.*	*n.s.*	*n.s.*

The starter fertilisation with NP resulted in a flowering anticipation of 1 day regardless LT fertilisation and year. This reduction in duration from sowing to flowering resulted in a slight reduction in grain moisture content at harvest (-0.7%) in 2019, only. However, neither the flowering date, nor the grain moisture at harvest did show any interaction effect of the starter fertilisation with LT management (Table 3).

4.4.4. Grain yield, yield components and quality traits

A significant effect of NP starter fertilization on grain yield and on its components was observed in Min-L and Min-H treatments, where grain yield raised of 1.3 and 3.2 t ha^{-1}, while KSM increased by 25 and 30%, respectively (Table 4; Fig.s 2g and 2h).

Table 4. Effect of long-term (LT) fertilisation, starter fertilisation and year on the grain yield, number of kernels per square meter (KSM), thousand kernels weight (TKW), grain protein content (GPC) and fumonisin (FBs) concentration. Means followed by different letters are significantly different (Bonferroni post hoc test) for each parameter. The level of significance, P(F), is shown in the table, n.s.: non-significant.

	Source of variation	**Grain yield (t ha^{-1})**	**KSM (n)**	**TKW (g)**	**GPC (%)**	**FBs (ppb)**
LT fertilization	Min-L	14.5 ab	4674	375 cd	8.8 a	5029
	Min-H	13.9 abc	4392	389 bc	9.4 a	4207
	Slu-L	12.9 c	4330	366 d	7.7 b	5500
	Slu-H	15.3 ab	4647	406 ab	9.1 a	8848
	Fym-L	13.8 bc	4515	384 cd	7.8 b	4253
	Fym-H	15.4 a	4550	409 a	9.2 a	6674
	P(F)	*0.001*	*n.s.*	*< 0.001*	*< 0.001*	*n.s.*
Starter fertilization	N	13.9 b	4399 b	388	8.7	5837
	NP	14.7 a	4637 a	389	8.6	5667
	P(F)	*0.009*	*0.031*	*n.s.*	*n.s.*	*n.s.*
Year	2019	13.4 b	4257 b	383 b	9.1 a	10250 a
	2020	15.2 a	4779 a	394 a	8.2 b	1254 b
	P(F)	*< 0.001*	*< 0.001*	*0.032*	*< 0.001*	*< 0.001*
LT × Starter	*P(F)*	*0.007*	*0.001*	*n.s.*	*n.s.*	*n.s.*
LT × Year	*P(F)*	*n.s.*	*n.s.*	*0.044*	*n.s.*	*n.s.*
Starter × Year	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
LT × St × Year	*P(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

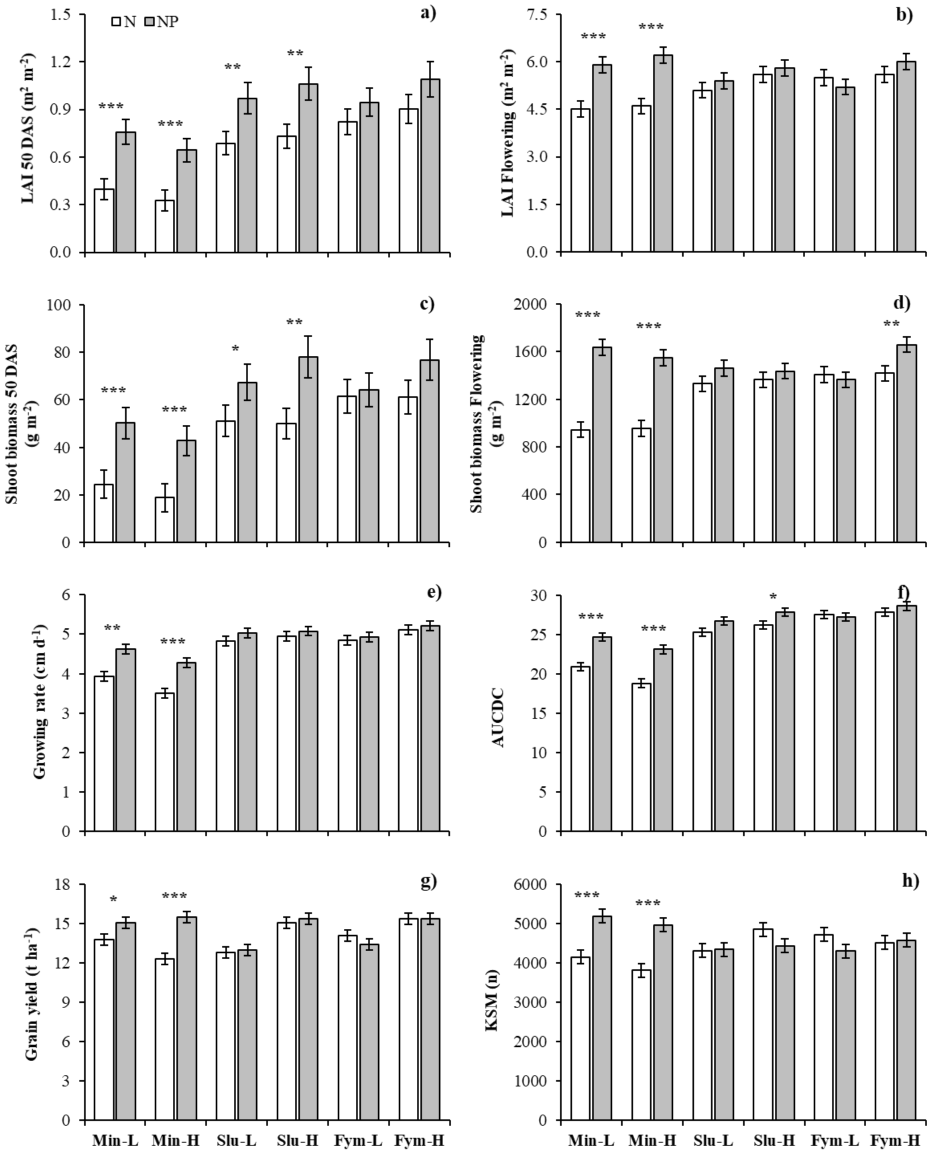

Figure 2. Effect of starter fertilisation with N (white histograms) and NP (grey histograms) on Leaf Area Index (LAI) at 50 DAS days after sowing (a) and flowering (b), shoot dry weight (SDW) at 50 DAS (c) and flowering (d), growing rate (e), area under the canopy development curve (AUCDC) (f), grain yield (g) and number of kernels per square meter (KSM) (h) within each long-term (LT) fertilisation. Asterisk demotes significant differences (significant at P <0.05; ** significant at P <0.01; *** significant at P <0.001) between means in LT fertilisation × starter fertilisation interaction, separated through a Bonferroni post hoc test. Error bars report ±standard error.*

No significant effect was recorded on grain yield in any of the manured treatments (Fym or Slu). Conversely, TKW was not influenced by starter fertilisation (Table 4).

None of the grain quality traits, protein content and contamination by fumonisin, was affected by starter fertilisation, but both were influenced by the year (Table 4). Grain protein content was higher in 2019, when yield was lower, but that year grain was also more contaminated by mycotoxins, due to wet conditions during the ripening phase.

4.4.5. Nutrient availability in the soil

Fig. 3 reports soil Olsen P (a) and nitrate N (b) concentrations as averaged over the two experimental growing seasons. Soil Olsen P was increased in the NP treatment compared to N in Min-H and Slu-H treatments, while this trend was observed in Min-L and Slu-L in only one of the experimental years. Conversely, when LT fertilisation included farmyard manure, no significant differences were found in soil Olsen P concentration as a consequence of NP starter fertilisation.

The nitrate-N concentration was highly variable over time and was not influenced by starter fertilisation. The highest values were recorded in Min-H.

The total N and P plant uptake, as indicators of soil nutrient availabilities at 50 DAS, flowering and harvest (Table 5), showed a significant effect of starter fertilisation and also a LT fertilisation × starter fertilisation interaction at all sampling dates (Table 6). When LT fertilisation included mineral fertilisers or bovine slurry, maize P uptake at 50 DAS was higher with NP starter fertilisation than N only. This behaviour was confirmed both at flowering and harvest in Min-L and Min-H, only, while starter fertilisation did not affect the P uptake at flowering and at harvest in all manured systems. In other words, initial positive effects of starter fertilisation were visible in systems with a lower initial P availability, but tended to decline during crop growth. Similarly, even N uptake showed higher values with NP starter fertilisation than N only at both rates of Min and Slu systems at 50 DAS, thus showing a synergic effect of the combined application of the two elements. However, differently to the P uptake, Fym-H recorded an increase of N uptake at 50 DAS when NP was applied at sowing. The N uptake of Min-L and Min-H treatment still differed at flowering, while at harvest only Min-H maintained a difference between the two starter fertilisation treatments.

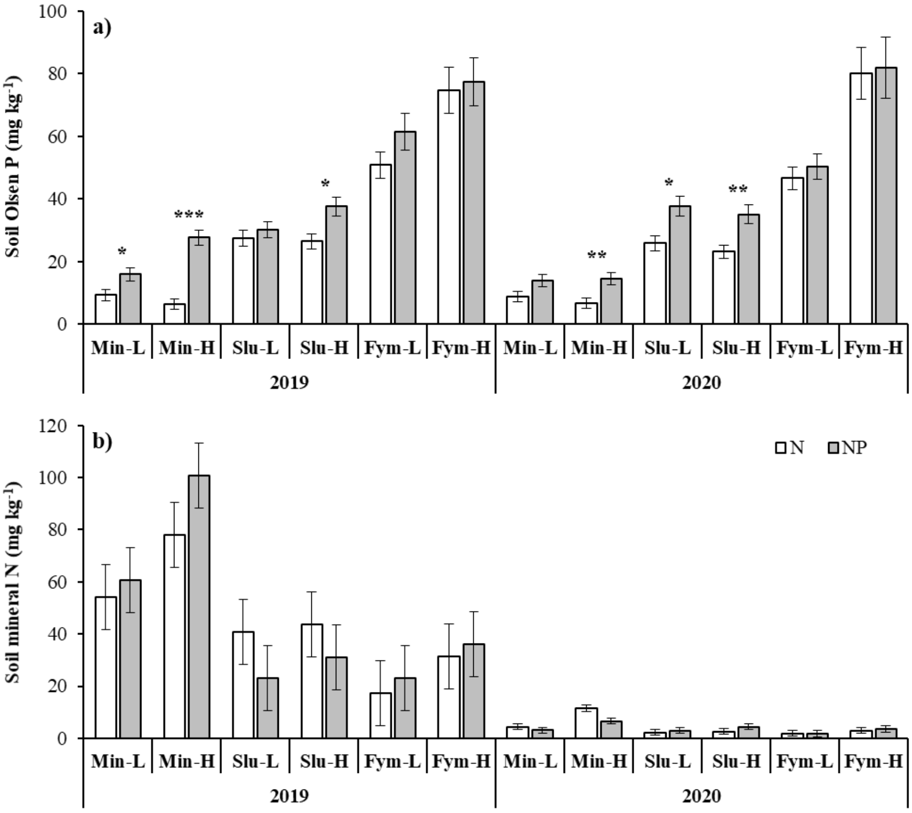

Figure 3. Effect of starter fertilisation with N (white histograms) and NP (grey histograms) on soil Olsen P (a) and mineral N (b) concentrations (mg kg^{-1} dry soil) measured in the first layer (0–30 cm) along the central maize rows during the two growing seasons and referred the different fertilisation strategies and fertiliser rates. Each value represents the average of three soil samplings occurred during the maize growth at 50 and 75 days after sowing and at crop flowering. Asterisk demotes significant differences (significant at P <0.05; ** significant at P <0.01; *** significant at P <0.001) between means in long-term (LT) fertilisation × starter fertilisation × year interaction (P= 0.010 and n.s. for Olsen P and mineral N, respectively), separated through a Bonferroni post hoc test. Error bars report ±standard error.*

The LT fertilisation management had significant effects on all enzymatic activities linked to P cycle (Table 7). Specifically, Fym applications led to a significant enhancement of all enzymatic activities compared to Slu and Min, and the latter generally showed the lowest values. Conversely, starter NP fertilisation was associated with reduced acid (acP) and alkaline phosphomonoesterase (alkP) as well and pyrophosphodiesterase (piroP) activities, if compared with N only. Since the soil pH was sub-alkaline, acid phosphatase was lower than alkaline phosphatase (Eivazi and Tabatabai, 1977).

Table 5. Effect of LT fertilisation, starter fertilisation and year on P and N plant uptake at 50 DAS, flowering stage and harvest. Means followed by different letters are significantly different (Bonferroni post hoc test) for each parameter. The level of significance, P(F), is shown in the table, n.s.: non-significant.

	Source of variation	**P uptake (kg ha^{-1})**			**N uptake (kg ha^{-1})**		
		50 DAS	**Flowering**	**Harvest**	**50 DAS**	**Flowering**	**Harvest**
LT fertilization	Min-L	1.4 b	31.2 bc	56.9 b	14.0 b	211	306 bc
	Min-H	1.1 a	29.5 c	53.0 b	12.3 b	231	343 ab
	Slu-L	2.6 a	40.4 ab	72.4 a	22.6 a	198	257 d
	Slu-H	2.6 a	38.6 ab	76.9 a	24.5 a	226	479 a
	Fym-L	3.0 a	42.8 a	79.1 a	23.1 a	203	281 cd
	Fym-H	3.4 a	46.8 a	82.1 a	27.0 a	238	489 a
	P(F)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*n.s.*	*< 0.001*
Starter fertilization	N	1.9 b	34.4 b	67.6 b	17.1 b	197 b	309 b
	NP	2.8 a	42.0 a	72.5 a	24.1 a	239 a	327 a
	P(F)	*< 0.001*	*< 0.001*	*0.012*	*< 0.001*	*< 0.001*	*0.022*
Year	2019	0.7 b	39.3	63.9 b	6.3 b	237 a	322
	2020	4.0 a	37.1	76.2 a	34.8 a	199 b	314
	P(F)	*< 0.001*	*n.s.*	*< 0.001*	*< 0.001*	*0.010*	*n.s.*
LT × Starter	*P(F)*	*0.029*	*0.021*	*0.017*	*0.008*	*0.001*	*0.021*
LT × Year	*P(F)*	*< 0.001*	*n.s.*	*< 0.001*	*< 0.001*	*n.s.*	*n.s.*
Starter × Year	*P(F)*	*< 0.001*	*n.s.*	*n.s.*	*< 0.001*	*n.s.*	*n.s.*
LT × Starter × Year	*P(F)*	*n.s.*	*n.s.*	*< 0.001*	*0.024*	*n.s.*	*n.s.*

Table 6. Effect of starter fertilisation with N and NP on P and N uptake at 50 DAS, flowering and harvest. Within each sampling date and LT fertilisation, different letters demote significant differences between starter fertilisation. Means followed by different letters are significantly different (Bonferroni post hoc test) for each parameter. The level of significance, P(F), is shown in the table 5.

	P uptake (kg ha^{-1})						**N uptake (kg ha^{-1})**					
	50 DAS		**Flowering**		**Harvest**		**50 DAS**		**Flowering**		**Harvest**	
	N	**NP**	**N**	**NP**	**N**	**NP**	**N**	**NP**	**N**	**NP**	**N**	**NP**
Min-L	0.9 b	1.9 a	23.1 b	39.3 a	51.5 b	62.4 a	8.5 b	19.5 a	157 b	265 a	293	318
Min-H	0.6 b	1.6 a	37.8 b	36.3 a	44.7 b	61.2 a	7.6 b	17.0 a	179 b	283 a	302 b	385 a
Slu-L	2.0 b	3.1 a	38.2	42.6	70.2	74.7	20.2 b	25.0 a	182	213	256	258
Slu-H	2.0 b	3.2 a	37.8	39.4	76.9	76.8	20.3 b	28.7 a	233	219	361	353
Fym-L	2.8	3.2	42.0	43.6	80.4	77.9	21.9	24.3	210	197	280	281
Fym-H	2.9	4.0	42.8	50.8	82.2	82.0	23.8 b	30.1 a	220	255	363	365

Table 7. Activity of acid (acP) and alkaline phosphomonoesterase (alkP), phosphodiesterase (bisP), pyrophosphodiesterase (piroP), inositol-P phosphatase (inositP) and nonanoate esterase (nona) expressed as nmol of 4-MUF (g dry soil)$^{-1}$ (hour)$^{-1}$ in Min-H, Slu-H and Fym-H system at 50 days after sowing over the 2019 growing season.

LT fertilization	Starter fertilization	acP	bisP	piroP	alkP	inositP	nona
Min-H	**N**	42	49	13	325	2	68
	NP	35	44	11	275	2	64
Slu-H	**N**	58	71	21	527	3	88
	NP	54	67	20	487	3	89
Fym-H	**N**	69	89	24	568	3	98
	NP	64	82	23	534	3	94
LT fertilization *P(F)*		*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.007*	*< 0.001*
Starter fertilization *P(F)*		*0.036*	*n.s.*	*0.029*	*0.027*	*n.s.*	*n.s.*
LT × Starter *P(F)*		*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

4.5. Discussion

The sub-surface application of NP fertilisers at sowing is a common agronomic practice in temperate maize growing areas, but it could be omitted in specific conditions. This study was performed to investigate the advantages of the application of NP starter fertilizer on maize growth, in systems characterised by different fertilisation managements - mineral fertilisers, bovine slurry or farmyard manure at two doses of N supply. At the start of the experiment, the six systems had different soil P contents, since they were the result of LT fertilisation managements, and P availability ranged from medium to very rich (Table 1), according to the Regione Piemonte (2021) classification. Likewise, soil organic matter concentration ranged from 1.2 to 1.9%, and a similar trend was observed for total N. Therefore, the six LT treatments allowed to study the effect of NP starter fertilisation in a relatively wide span of situations differing for the overall fertility, on the same soil. Another important source of variation, that highlighted significant interactions with starter fertilisation, was the weather. The two observational years were characterised by different trends in temperature, in particular in the first growing phases of the crop. Therefore, interactions between starter fertilisation and LT fertilisation management and with the year are the most relevant results of this work.

The starter fertilisation with NP was confirmed to enhance early crop growth, coherently with several studies that document that P is the second key limiting nutrient for maize growth, after N (Plénet et al., 2000a; Plénet et al., 2000b; Grant et al., 2001). Differences were more marked in systems where mineral fertilisers only were used, than in manured ones, Fym or Slu. The cause for this could be simply the fact that soil available P was very different in the observed treatments, and benefits were reduced where a greater soil available P could ensure a good nourishment to the crop. However, a positive effect of starter fertilisation was also observed in systems where the soil P status could indicate that an extra supply was not necessary.

At all systems, differences in crop growth indicators that were detectable in early stages between NP and N treatments, in particular LAI and shoot biomass, progressively reduced until the flowering stage, and became non-significant or negligible in manured systems. However, the enhanced early vigour due to NP significantly anticipated flowering by 1 day in all LT fertilisation systems. This observation confirmed what was found by other authors, as similar anticipation of anthesis was reported for example by Kaiser et al. (2016).

At harvest, NP starter fertilisation induced a higher grain yield than N only, at both rates of Min systems, while no differences were recorded in any manured system. The absence of an effect in maize grain yield due to starter fertilisation in P-rich soils, although the effect was evident in crop growth at early stages, agrees with findings by Bordoli and Mallarino (1998), Rehm and Lamb (2009), Kaiser et al. (2005), and Kaiser et al. (2016). In contrast, the increase in grain yield following NP starter fertilisation recorded in Min could be due to boosted LAI at tassel emission, and consequent higher photosynthetic rate, that led to a higher number of KSM and a decrease in barren ear tip lengths. This was coherent with findings of Zhang et al. (2018), who indicated a LAI value at flowering of ~4.8 m^2 m^{-2} as critical, and values measured in this study were 4.5 and 4.6 m^2 m^{-2} in Min-L and Min-H N, respectively. Andrade (1995) also reported that the reduction of crop growth rate determined by a limited radiation interception during flowering was responsible for small number of grains.

The second important variable that affected plant response to NP fertilisation was air temperature in the first growing stages. Low temperatures recorded in April and May 2019 strongly depressed plant growth, but NP starter fertilisation significantly reduced the negative impact of unfavourable weather conditions. Wortmann et al. (2006) and Kaiser et al. (2016) reported that the extent of maize response to starter fertilization is significantly influenced by environmental conditions such as meteorological trend, soil texture and their effects on soil moisture and temperature. Past research has shown that plant P requirement is higher with cool weather conditions during seedling (Ketcheson, 1957), as root growth is reduced and this adversely impacts the uptake of nutrients, especially P, which is scarcely mobile in the soil and must be intercepted by roots (Wang et al., 2016). Phosphorus promotes root growth, and Zhang et al. (2012) showed that maize root length in a 44 kg P ha^{-1} treatment was significantly greater than that in a no-P treatment in the 50–60 cm soil layer at maize flowering. However, our experimental setup does not allow to test the effect of single P supplies.

In the year characterised by cold temperatures in early growth stages, 2019, maize could compensate a slow establishment with a fast development in the second growth phase, that led to limited differences among treatments at flowering. The temperature trend was reversed in 2020, when growth conditions were more favourable in the first phase but colder in the second phase. Although less pronounced effects on maize early vigour due to NP starter fertilisation were recorded in the latter case, the maize anthesis was anticipated in all systems by the same extent recorded in 2019. Similarly, even LAI and shoot dry weight still showed differences at flowering in mineral-fertilised systems.

Another important period whose weather affects yield amount and quality is the ripening phase, that in our study had a further levelling effect on starter fertilisation treatments. The grain moisture content at harvest was significantly decreased by NP fertilisation during the 2019 growing season, only. All the other quality and sanitary traits of harvested grain seemed more influenced by N fertilisation level (higher protein content was obtained at higher N rates, owing to a quicker availability of N to plants; Saha et al., 2008); or by the meteorological circumstances. In particular, the grain fumonisin contamination was larger in 2019, probably because of wetter conditions in September. It is in fact known that *Fusarium verticillioides*‘s growth and biosynthesis of FBs are maximised in warm and wet air conditions (Sancho et al., 2018).

An unexpected finding of this work was that starter P fertilisation was effective in first growth stages also in systems where the soil P test had evidenced a high availability. There are two possible explanations for this. One calls the synergic effect of N and P additions on crop early growth. A growth promotion was in fact observed when both nutrients were supplied, but not when N only was supplied. Duncan and Ohlrogge (1958) reported that a rapid development and branching of roots are promoted by combined N and P addition, but not by N only. The synergic addition of N and P has therefore a stimulating effect on maize roots that further helps soil nutrients exploitation. The starter fertilisation also affected the expression of soil enzymes connected with P cycle. In particular, NP addition at sowing caused a general reduction of enzymatic activities at 50 DAS, thus confirming findings by Nannipieri et al. (2010), who stated that soil phosphatase activity generally decreases in response to mineral P fertiliser application. However, this is not always true, as, for instance, Margalef et al. (2021) found that applying both N and P to soils with low P availability had a positive effect on phosphatase activity, whereas it decreased when N and P were applied to soils with high natural P contents. In contrast, the experiment here described did not show any interaction between starter and LT fertilisation, and the overall reduction of P-related enzymes was observed in all treatments. The LT fertilisation was a great determinant in the P-related enzymes, as the largest enzyme concentrations were found when Fym was used, probably as a consequence of a larger substrate availability. The contrasting behaviour of P enzyme concentrations, that increased across LT systems with increasing soil P test availability, but decreased after starter fertiliser P supply, remains unexplained.

A second hypothesis to interpret the unexpected positive effect of NP fertilisation also in P-rich systems is instead linked to the effectiveness of soil P test in identifying critical levels of P concentrations for crop growth. This held true also when Olsen P was measured close to roots (as in this study) and not on bulk soil (as normally done). Literature reports many studies aimed at identifying critical available P levels for maize, and values ranged from ~5 to 40 mg kg^{-1} (Mallarino and Blackmer, 1992; Wen et al., 2016; Wu et al., 2020), with differences depending on cropping system (continuous or rotational maize) and soil type (Khan et al., 2018; Xi et al., 2016; Bai et al., 2013). Our data indicate a critical soil concentration at quite high values of the soil P test. In fact, the total plant uptake at harvest - a true indicator of availability - showed a linear-plateau response to soil Olsen P measured near roots at 50 DAS (Fig. 4). The plant P uptake increased linearly up to the threshold of 39 mg P kg^{-1} of Olsen P, then stabilised, as luxury consumption of P is not typical in maize. Wu et al. (2020) also observed a limited plant growth increase due to an additional P application, when soil P availability was higher than a critical value of 40 mg kg^{-1}. Results reported in Fig. 4 indicate that both Min and Slu systems were in the range of soil P test values where a positive effect of fertilisation was expected. The moderate, but positive effect of starter P fertilisation in Fym systems could then be due to a temporary immobilisation of mineralised P in microbial biomass (Jones and Oburger, 2010), but it did not affect the overall plant uptake. The threshold below which an addition of P was effective in increasing P availability was therefore rather high in our conditions, about 39 mg kg^{-1} of soil Olsen P.

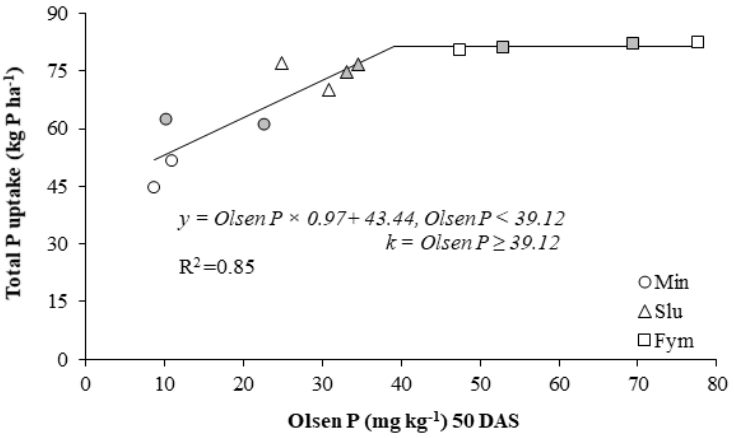

Figure 4. Total above-ground plant P uptake at harvest in response to the soil Olsen P concentration at 50 days after sowing (DAS), averaged over the two years (n=6). Open symbols represent N starter fertilisation, while closed symbols show NP starter fertilisation.

The optimal dose of mineral fertilisers is a compromise between maximum crop benefit and drawbacks such as economical cost and environmental risk, that is particularly high for P (Schoumans et al., 2015; Garske et al., 2020; Stamm et al., 2021). Therefore, provided that plant uptake capacity should not be exceeded in order to avoid soil overburden, there is a need to find the right dose that minimises both the risk of dispersing P into the environment and the risk of reducing crop nutrient use efficiency in case of adverse weather conditions. The limited advantages observed in this study in terms of crop growth, anticipation of flowering date, and reduction of grain moisture at harvest could be negligible in years with a normal weather, but could be crucial in ensuring yield amount and quality in adverse weather years. In a context of climate change, this might become relevant soon.

4.6. Conclusions

The combined application of N and P fertilisers at maize sowing improved early crop growth and increased grain yield when applied to systems where fertilisation included mineral fertilisers only and the soil P status was medium. On the other hand, in systems where long-term farmyard manure applications had increased the soil Olsen P availability to high levels, benefits due to starter NP fertilisation were relevant in early crop growth, but not visible at harvest. Systems fertilised with bovine slurry were intermediate, and again showed positive effects of NP starter fertilisation in early growth but not in yield.

The crop P uptake increased with increasing soil P status up to the critical value of 39 mg kg^{-1} of soil. Environmental measures set a threshold above which P fertilisation should be suspended in order to better exploit soil P legacy. This value is 25 mg kg^{-1} in the regional legislation and in other European countries (Amery and Schoumans, 2014), or even lower (15 mg kg^{-1} in Colorado and Idaho in USA; Sharpley et al., 2002), sometimes depending on crop type (for example 25 or 45 mg kg^{-1} in Northern Ireland), Our study provides evidence that the threshold value above which no P should be supplied does not maximise the maize nutrient status. A limited addition of mineral P combined with N at sowing could be useful to boost early maize growth in temperate climates, even in a context of urgent environmental protection concerns. In uncertainties of a rapidly changing climate with increased fluctuations of temperature and rainfall, the adoption of NP starter fertilisation at maize sowing may partially alleviate the negative effects of early cold stress, emphasising the role of this management practice in ensuring stable yields.

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4.7. Supplementary information

Table S1. Chemical properties of bovine slurry (SLU) and farmyard manure (FYM) used for the field experiment.

5		DM[a]	N[b]	$N\text{-}NH_4$	P	K	C
		%	kg t^{-1} DM				
2019	**SLU**	5.7	59.3	25.5	2.8	28.7	405.1
	FYM	21.6	34.9	0.3	4.4	33.7	365.4
2020	**SLU**	4.8	64.5	29.9	11.3	33.9	369.5
	FYM	19.2	32.0	4.2	5.3	25.5	359.0

[a] weight at 105 °C
[b] Kjeldahl method

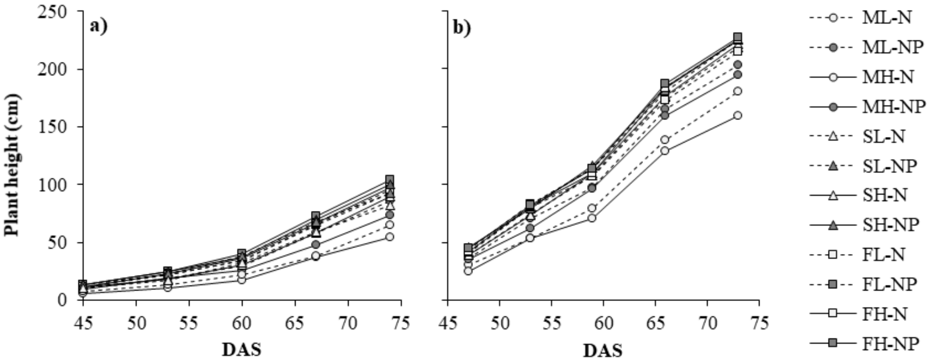

Figure S1. Effect of the starter fertilisation strategies with N (open symbols) and NP (close symbols) on the maize canopy development, expressed as plant height measured during leaf emission and stem elongation stages over the 2019 (a) and 2020 (b) growing seasons.

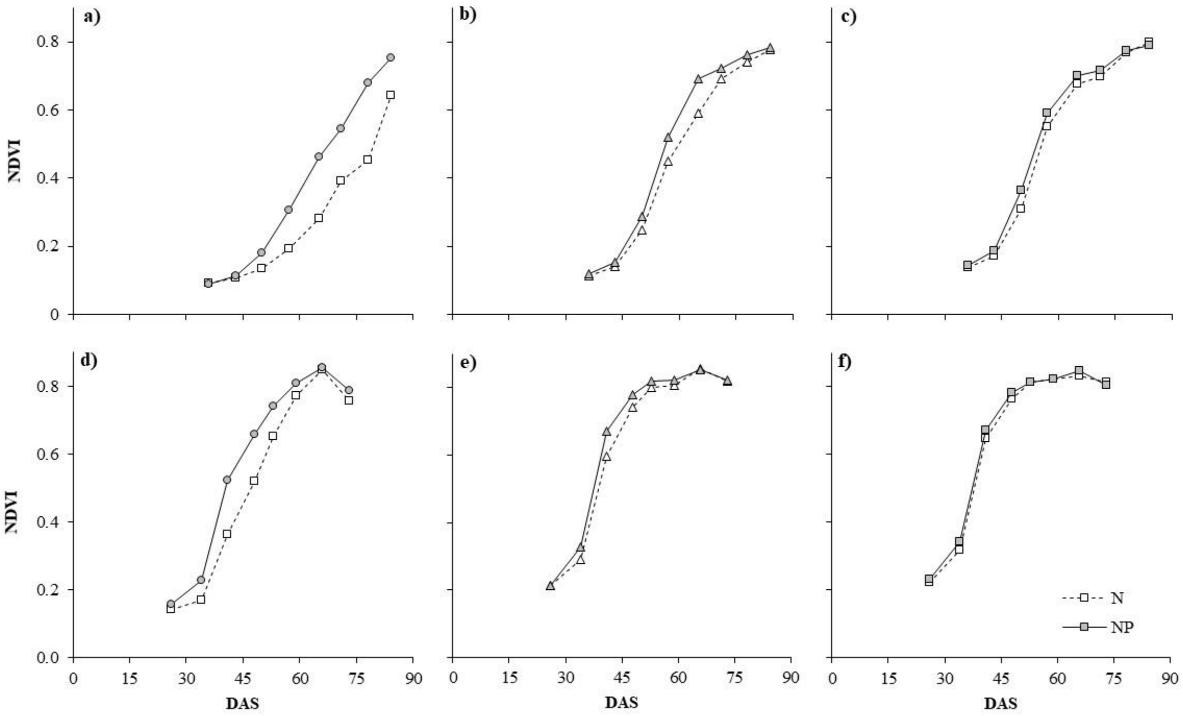

Figure S2. Effect of the starter fertilisation strategies with N (white symbols and sashed line) and NP (grey symbols and solid line) on the maize canopy development, expressed as NDVI from the 3-leaf stage to the tassel emission of Min-H (a and d; circle symbols), Slu-H (b and e; triangle symbols) and Fym-H (c and f; square symbols) systems during the 2019 (a, b and c) and 2020 (d, e and f) growing seasons.

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5. Maize response to localized mineral or organic NP starter fertilization under different soil tillage methods

Authors: Michela Battisti[1], Laura Zavattaro[2], Luca Capo[1], Massimo Blandino[1*]

Affiliation:

[1] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy

[2] Department of Veterinary Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy

**Corresponding author:* Massimo Blandino, Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy.

E-mail: massimo.blandino@unito.it

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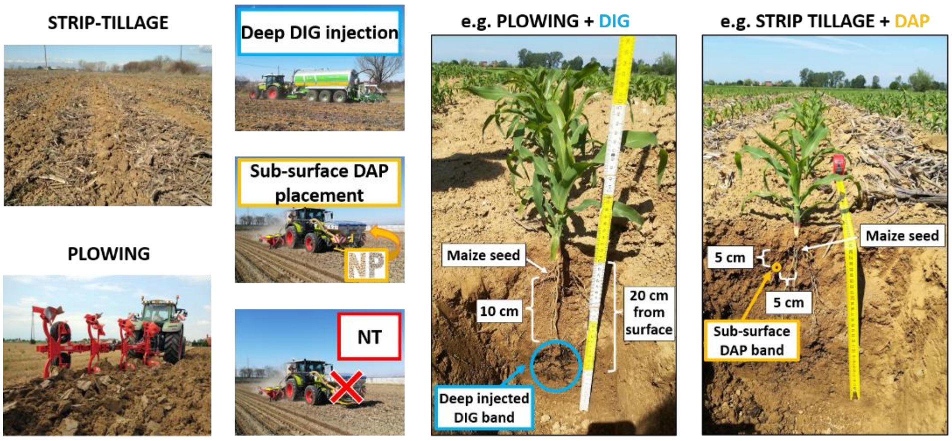
Graphical abstract



5.1. Abstract

An early maize (*Zea mays* L.) sowing date plays a key role in increasing the grain yield, especially in a temperate climate. The adoption of reduced tillage techniques, such as strip tillage (ST), can decrease cultivation costs, compared to plowing (PLOW), but might also reduce the crop early development rate as a result of lower soil temperatures. Therefore, farmers often adopt starter fertilizations when sowing maize, in order to improve the early-season nutrient uptake, nutrient use efficiency and plant development. The aims of this study were to assess the effects of the adoption of ST, compared to PLOW, and a starter fertilization with deep-injected digestate (DIG) or sub-surface placed diammonium phosphate (DAP), compared to an unfertilized treatment (NT) on the early growth, grain yield and grain qualitative and sanitary traits of maize. Field experiments were carried out in NW Italy over the 2019 and 2020 growing seasons on two different soils. ST determined cooler soil conditions at both sites, compared to PLOW, thus resulting in lower NDVI values due to both slower plant development and lower final crop density. The starter fertilization with DAP recorded the best early vigor and canopy development, expressed as plant height in both sites and for both tillage techniques, while the DIG values were intermediate between the DAP and NT treatments. Although the effects on the early vigor of maize differed between the experimental sites, displaying more pronounced differences in the finer-textured soil, ST on average delayed the flowering (+2.2 days) and consequently delayed the moisture dry down process (+1.2% grain moisture content at harvest), compared to PLOW. The starter fertilization with DIG or DAP led to an earlier flowering, compared to NT (-2.6 and -4.6 days, respectively). The grain yield was not affected by the tillage system, as the plants in ST compensated for the lower plant density with an increased ear length (+1.5%). Instead, DIG and DAP recorded similar and higher grain yields (+1.8 and +1.6 Mg ha^{-1}) than NT. DIG application led to a higher grain protein content and lower level of fumonisin contamination than the other treatments, while ST determined higher deoxynivalenol values than PLOW. The combination of ST and DIG as a starter fertilization can help maintain the production level and the grain quality, compared to conventional techniques that involve the use of mineral fertilizers and plowing, thereby leading to a higher sustainability of the maize cropping system.

Keywords: reduced tillage, phosphorus, nitrogen, starer fertilizer, yield quality, mycotoxins, early growth

5.2. Introduction

Maize (*Zea mais* L.) is the crop with the largest global production, and it is used for both food and feeds as well as for industrial or energy purposes. Early planting plays a key role in achieving the full potential yield in temperate growing areas as it leads to an increase in the length of both the vegetative period and the reproductive phase (Waqas et al., 2021), thus promoting higher yields and higher resource use efficiencies. Farmers sow maize early to escape heat and drought stress during the reproductive phase (Waqas et al., 2021) obtaining an early development and an earlier flowering, which in turn leads to a higher radiation interception and a greater number of kernels per surface unit and a faster growth rate during ripening (Sacks and Kucharik, 2011; Islam et al., 2015), an increase in grain yield (Salvagiotti et al., 2013) and a lower mycotoxin content (Blandino et al., 2009a). In addition to the sowing time, other crop practices, such as tillage and use of starter fertilization, could also have direct influence on the maize ability to overcome a delayed development in early stages, when low-temperature stress may occur, thus yielding earlier flowering and associated benefits.

Although the adoption of no tillage or minimum tillage has also increased globally for maize (Kassam et al., 2019), these techniques are still a matter of concern since they have cooler temperatures and higher moisture contents in the early crop growth stages (Blanco-Canqui and Ruis, 2018). All these conditions may negatively influence the critical phase of crop establishment, and may result in a delay in emergence and slower early growth (Licht and Al-Kaisi, 2005). One strategy to overcome this problem is to apply tillage to only a part of the surface, which could help to reduce the adverse effects of no-tillage, and maintain most of its positive characteristics. With this technique, a narrow strip is tilled and prepared as a seedbed with a single passage that also includes the displacement of previous crop residues and the incorporation of fertilizers. The limited tilled areas in the strip tillage (ST) are associated with warmer soil temperatures and lower bulk densities than no-tillage (Celik et al., 2013). Licht and Al-Kaisi (2005) reported an increase of 1.2-1.4 °C when the soil was strip-tilled, compared to no-tillage, and the plant emergence rate was similar to a chisel plowed treatment. On a sandy loam soil subjected to moldboard plowing or strip-tillage, Vyn and Raimbault (1992) also recorded the same maize plant density. Many studies have evaluated the effects of conventional, strip- and no-tillage techniques on maize yield. Liu and Wiatrak (2012) reported that the maize yield was similar under plowing and ST, but significantly lower under no-tillage, in a loamy sand soil in a dryland rain-fed environment. Conversely, Vyn and Raimbault (1992) recorded a 10-12% reduction in grain yield under ST in Ontario (Canada), compared to plowing, in both silty loam and clayey loam soils. Some authors have instead found an increase in yield under strip tillage, compared to a conventional system. In tropical growing areas, Mullins et al. (1998), for example, observed a yield increase of 16% of silage and 43% of grain in maize for silage and grain production cropping systems, respectively. Furthermore, in terms of economic costs and profitability, farmers who adopt ST have a 34% greater net return than those who apply conventional tillage (i.e. plowing followed by secondary tillage), mainly due to the reduced number of operations, and the consequent reduced time consumption, fuel consumption and machinery investment costs (Morris et al., 2010; Deleon et al., 2020). Moreover, the reduced number of preparatory operations could favor early sowing dates, thereby guaranteeing an optimal timeliness in crop establishment (Morris et al., 2010). However, the occurrence of previous crop residues on the soil surface as a result of reduced tillage is a critical key factor in the inoculum production of fungal species, and an accumulation of mycotoxins has been observed in wheat and other winter cereals (Blandino et al., 2012). Nevertheless, the role of reduced tillage on the content of such contaminants in maize grain is still unknown.

On the other hand, the initial growth of maize could benefit from localized fertilization. The precise placement of phosphorus (P) and nitrogen (N) fertilizers at sowing as a starter fertilization is a common practice used by maize farmers to improve the early-season nutrient uptake and early development of the plant, but also the total nutrient use efficiency, and to achieve adequate yields, in particular in regions where early temperatures are suboptimal for maize growth (Nkebiwe et al., 2016; Quinn et al., 2020). Many studies carried out under plowing conditions have reported benefits for the early growing of maize due to the synergism of P with N, especially as ammonium (NH_4^+), if supplied in sub-surface bands near the seed furrows. The main benefits include lateral and fine root proliferation (Jing et al., 2010; Ma et al., 2013), increased P and micronutrient availabilities, due to a lower rhizosphere pH following NH_4^+ uptake (Jing et al., 2012; Ma et al., 2014), and boosting of early crop development (Osborne, 2005; Ma et al., 2015). Positive effects have also been found in soils with a medium or high available P content (Blandino et al., submitted; Kaiser et al., 2016). In a context of sustainable agriculture, inspired by circular economy principles, i.e., nutrient recycling and recovery to avoid waste creation, manufactured P and N fertilizers could be replaced by animal manures or other organic materials. Animal manures are historically used for crop fertilization purposes in regions characterized by an integrated livestock-mixed agriculture, but their repeated supply often causes an enrichment of the soil P status, due to the lower N/P ratios of manures than that of plants, with possible threats to the quality of freshwater (Borda et al., 2011). Although the organic P fraction contained in manures is not immediately available for plant uptake, soluble organic phosphate compounds and organic acids are released after mineralization. These molecules prevent P fixation in soil and result in greater concentrations of available P (Laboski and Lamb, 2003). A review by Kratz et al. (2019) showed that farmyard manure and slurry contain a large amount of easily soluble P, thus displaying a medium-to-high P fertilizing value compared to mineral fertilizers. This was also observed for biogas digestates, which often contain animal manures as the main or co-substrate for digestion. Furthermore, Battaglia et al. (2021) demonstrated that manure injection is also compatible with both no- and minimum-tillage, if associated with precision auto-guidance systems which allow a simultaneous injection below the future seed rows with the tillage operation (Tauchnitz et al., 2018). The adoption of liquid manure injection along seed rows as a starter fertilization to replace mineral fertilizers can help increase the sustainability of agriculture and implement fertilization best management practices based on the 4Rs principles (right nutrient source, right rate, right time and right place).

The effects of the liquid manure injection in a band close to maize rows as an alternative N and P source to replace mineral starter fertilizers on the soil macro- and micronutrient availability and consequently on the nutrient use efficiency and on the final yield of maize silage have been tested in several studies carried out in Germany (Westerschulte et al., 2018, Federolf et al., 2016; Federolf et al., 2017), Denmark (Pedersen et al. 2020a), the Netherlands (Schröder et al. 2015), the USA (Battaglia et al., 2021) and Canada (Hunt and Bittman, 2021). However, no studies have evaluated the effects of manure injection as a starter fertilization and its combination with reduced tillage techniques on maize grain yield. None of the aforementioned studies included measurements of the early growth of plants or the date of flowering, the associated grain yield or the qualitative benefits; the effects of starter fertilization, in a minimum tillage context, therefore still remain unclear.

The objective of the present study has been to quantify the effects of the adoption of ST, compared to plowing, and of localized fertilization at sowing with biogas raw digestate or mineral NP fertilizer, on the early development, growth, yield and sanitary traits of maize, in two different kinds of soils, under the hypothesis that a localized manure distribution could efficiently surrogate mineral NP fertilizers in promoting the early growth of maize, in particular in a context of reduced tillage techniques.

5.3. Materials and methods

5.3.1. Study sites

The study was performed in field experiments at Carmagnola (44°23’N, 7°40’E) and Poirino (44°56’N, 7°51’E), in North-West Italy, during the 2019 and 2020 growing seasons. Both soils lie in a plain area which is intensively cultivated. The soil texture was loamy silt at both locations, but the soil at Carmagnola was richer in sand and poorer in clay than the Poirino soil (Table 1).

Table 1. Main physical and chemical characteristics of the topsoil (0-30 cm) of the Carmagnola and Poirino soils.

Parameters	**Measurement units**	**Carmagnola**	**Poirino**
Soil type[1]		Typic Udifluvent	Fluventic Haplustepts
Sand (2 -0.05 mm)	%	35.5	25.7
Silt (0.05 - 0.002 mm)	%	57.9	61.8
Clay (< 0.002 mm)	%	6.6	12.5
pH		8.1	6.3
Total $CaCO_3$	%	1.2	-
Organic matter	%	2.3	1.5
C/N		8.0	9.6
Cation Exchange Capacity (C.E.C.)	meq/100g	12.0	13.9
Exchangeable K	mg kg^{-1}	174.0	82.0
Total Nitrogen			
2019	%	0.163	0.089
2020	%	0.079	0.085
Olsen P			
2019	mg kg^{-1}	14	18
2020	mg kg^{-1}	21	58

[1] Soil Survey Staff, 2014

The soil pH was medium alkaline (8.1) at Carmagnola and slightly acid (6.3) at Poirino. The available P content (Olsen et al., 1954) was medium in both plots used in the two growing seasons (14 and 21 mg kg^{-1}) at Carmagnola, while it was medium (18 mg kg^{-1}) in 2019 and high (58 mg kg^{-1}) in 2020 at Poirino. The total N in the soil was medium at Carmagnola in 2019 and low for all the other site-year combinations. The amount of organic matter was medium at both sites (2.3 and 1.5% at Carmagnola and Poirino, respectively) and so was the cation exchange capacity.

The climate was temperate, sub-continental at both sites, with two main rainy periods concentrated in spring and autumn. Daily temperatures and precipitation were measured by meteorological stations located near the experimental sites. Table 2 reports the monthly rainfall and the growing degree days (GDDs) relative to the two experimental seasons.

Table 3. Cumulative monthly rainfall and air growing degree days (10°C base; GDDs) during the maize crop cycle for the 2019 and 2020 growing seasons at the Carmagnola and Poirino experimental sites.

	GDDs (°C- day)				**Rainfall (mm)**			
	Carmagnola		**Poirino**		**Carmagnola**		**Poirino**	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
March	115	54	120	64	3	13	6	26
April	114	146	123	160	86	91	85	86
May	167	248	185	266	72	93	103	98
June	344	295	370	318	25	129	25	167
July	412	395	432	420	119	37	120	36
August	400	402	426	415	79	115	86	91
September	265	281	287	232	92	29	63	5
Total	**1817**	**1821**	**1943**	**1874**	**476**	**508**	**488**	**509**

5.3.2. Experimental set up

Different soil tillage and starter fertilization strategies were compared in each site and for each year according to a full factorial combination. The field experiments utilized a factorial arrangement within a split-plot design, where the tillage system represented the main plot and the starter fertilization was the subplot. Subplots were 30 × 3 m, replicated four times. In 2020, the experiment was performed in a new area adjacent to the one used in 2019 at both sites, to avoid carry-over effects due to treatments.

5.3.3. Tillage techniques

The strip tillage technique (ST) was compared with a reference plowed system (PLOW) for the seedbed preparation. ST was performed the day before maize sowing with combined equipment, which consisted of a four-row tiller (3 m width; Strip Hawk Easy, MOM, Villa Fontana, Verona, Italy) set at a depth of 25 cm that tilled 25 cm strips and left 50 cm of standing crop residues between the tilled rows. Each working unit consisted of a sequence of cutting discs followed by star-shape discs for the row cleaning and a C-shank with tines for a deeper soil tillage, plus a couple of side-closing discs that contained the clods within the strip. A final chain roll broke the clods and levelled and firmed the seedbed. The reference tillage technique in the PLOW treatment involved soil plowing at a depth of 30 cm, followed by disc harrowing and rotary tiller levelling, which represent the traditional sequence of operations commonly used by farmers in the area. The soil plowing and the following secondary operations were performed in spring within a week preceding maize sowing.

The previous crop was maize for grain at both sites, as maize continuous mono-cropping is typical in Northern Italy, and the maize stovers, after an autumn chopping, were left on the soil surface until tillage. No additional field operations were performed before maize sowing in the ST system.

5.3.4. Starter fertilization strategies and nutrients management

A deep-placed digestate fertilization (DIG) was compared with mineral fertilization (DAP) sub-surface placed at sowing and with a control (NT). The DIG and DAP treatments received the same -P starter fertilization at sowing, but from different sources. The DIG treatment used digestate originating from the anaerobic digestion of farmyard manure (75%) and maize (25%) to supply 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1}. The digestate was injected, concurrently with the strip tillage operation, or after rotary harrowing, as a deep band, at a depth of ~20 cm from the soil surface by a slurry tank equipped with injector tools. The digestate contained 66.5 g kg^{-1} of N (51% of which as N-NH_4), 24.1 g kg^{-1} of

P_2O_5, and 75.4 g kg^{-1} of K_2O, all expressed on a dry matter basis, while its pH was 8.2.

Diammonium phosphate (18% N and 46% P_2O_5) was distributed in the DAP treatment to supply 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} in sub-surface bands, 5 cm apart from the maize seed rows, using a calibrated granular dispenser mounted onto the precision seeder.

The control treatment NT did not receive any N or P fertilizer at sowing. This fertilization strategy is commonly applied in the growing areas in soils where P does not represent a crop constraint; it consists in applying all the crop N requirements at seven-leaf stage, and was included as a control to measure benefits of the main treatments - including fertilization at sowing - on the early crop growth, according to Kaiser et al. (2016) and Osborne (2005).

The pre-sowing fertilization management provided 100 kg ha^{-1} of K_2O as potassium chloride (60% K_2O *w/w*) broadcast applied, albeit only to the NT and DAP treatments.

In order to cope with the N crop needs, a top-dressing fertilization with urea was carried out at approximately GS 17 (according to the BBCH scale; Table 3) without soil incorporation to maintain a similar management between the tillage treatments (Table S1). In order to minimize ammonia volatilization, urea was side-dressed using a pneumatic fertilizer spreader after a rainy day and in the late afternoon. The N requirement was calculated separately for each experimental site taking account the different soil fertility levels and local standards to achieve the full potential grain yield. The top-dressing N rates applied as urea were obtained as the difference between the expected crop needs and the amount of N supplied with the starter fertilization for the DAP and DIG treatments, while the NT treatment received the same top-dressing N rate as the DAP treatment (Table S1).

Table 3. Maize hybrids, relative maturity FAO class, sowing, top-dressing fertilization and harvesting dates for each site-year combination.

Site - Year	Hybrid	FAO Class	Sowing	Top-dressing fertilization	Harvesting
Carmagnola - 2019	Korimbos (KWS Saat AG)	500	04th April	30th May	25th September
Poirino - 2019	Korimbos (KWS Saat AG)	500	21st March	03rd June	24th September
Carmagnola - 2020	LG 30600 (LG Seeds, Limagrain)	600	08th April	22nd May	23rd September
Poirino - 2020	Sy Brabus (Syngenta)	700	31st March	27th May	24th September

5.3.5. Field operations and crop management practices

The maize hybrids, the relative maturity FAO classes, and the sowing and harvesting dates are reported in Table 3. The distance between the plants was 16 cm, thereby providing a theoretical crop density of 8.3 plant m^{-2}. An RTK GPS-based guidance was employed to obtain an accurate seed placement along the central line of either the ST or the digestate bands. The planter was also carefully calibrated in order to obtain the same sowing depth in both tillage systems.

The weed control of the ST plots involved the use of glyphosate (1.92 kg Active Ingredient (AI) ha^{-1}) (Roundup® Platinum, Bayer CropScience S.r.l., Milan, Italy) before tillage. The weed control was performed at pre-emergence with mesotrione (0.15 kg AI ha^{-1}), S-metolachlor (1.25 kg AI ha^{-1}) and terbuthylazine (0.75 kg AI ha^{-1}) (Lumax®, Syngenta Crop Protection S.p.A., Milan, Italy) under both tillage conditions and in both locations. Furthermore, the ST plots in Poirino also required a post-emergence weed control, using Nicosulfuron (40.5 g AI ha^{-1}), Rimsulfuron (10.1 g AI ha^{-1}) and Dicamba (0.24 kg AI ha^{-1}) (Principal® Mais, Du Pont de Nemours Italiana S.r.l., Milan, Italy).

In order to avoid any drought stress until the physiological maturity, irrigation was performed, according to the management commonly used by farmers in the area, with a drip irrigation hose system at Carmagnola (twice a week with 15 mm from the beginning of July to the of August for both years) and using the sprinkler method at Poirino (once per year in July with 40 mm).

In order to evaluate the role of the presence of maize residues on the soil surface as ear rot inoculum, the control of European Corn Borer (*Ostrinia nubilalis* Hübner; ECB) was conducted by applying Chlorantraniliprole (0.03 kg AI ha^{-1}) and Lambda-cialotrina (0.01 kg AI ha^{-1}) (Ampligo ®, Syngenta Crop Protection S.p.A., Milan, Italy) as a foliar insecticide at the maize milk ripening stage (GS75).

5.3.6. Soil temperature, plant emergence and early crop vigor measurements

A series of measurements on the soil and the crop were made on the two central rows of each plot. The soil temperature was measured during the crop emergence and seedling stages using a 10K NTC Thermistor sensor (Tinytag® TGP-4017 temperature logger devices with hourly record frequency) placed at a depth of 7 cm along the seed rows. Maize emergence was assessed twice by measuring the number of emerged plants per square meter, when approximately 50% and 100% of the potential plants had emerged in the PLOW reference treatment.

The Normalized Difference Vegetation Index (NDVI) was measured during the vegetative stages by means of a hand-held optical sensing device GreenSeekerTM® (Trimble©, Sunnyvale, California, USA). An operator held the instrument approximately 60 cm above the maize canopy and walked at a constant speed along the entire length of the plot. This assessment was performed every week from the three-leaf stage (GS 13) until tassel emission (GS 55). The plant growth rate was calculated as the average daily NDVI increase during the vegetative period, from the 3-leaf emission stage to the middle of stem elongation. As detailed by Capo et al. (2020), the NDVI observations were used to calculate the Area Under Canopy Development Curve (AUCDC) using the following formula:

$$AUCDC = \sum_{i}^{n-1} \{[(R_i + R_{i+1})/2]\,(t_{i+1} - t_i)\}$$

where R is the NDVI value, t is the time of observation expressed in days and n is the number of measurements.

The plant height was recorded between the leaf emission (~GS 15) and the stalk elongation (~GS 33) stages on 20 plants, from the ground level up to the collar of the uppermost fully-developed leaf or up to the uppermost detectable node, respectively. The anthesis date was determined when >50% of the plants in each plot showed the stigmata tips (GS 63) and was expressed as days after sowing (DAS). The stalk diameter was measured in correspondence of the first internode during the ripening stage with a caliper to calculate its area, and the plant height with a folding ruler, as a proxy of plant stability. At maturity plant height was measured from the ground up to the uppermost leaf collar.

5.3.7. Grain yield and yield components

Maize grain yield and components, fully developed ears, plant density and number of barren plants were assessed on 4.5 m^2 areas in the central part of each plot, at harvest maturity. The grain yield components included the cob length, as measured on a sub-sample of 15 ears, the number of kernel rows and the number of kernels per row, both of which were determined on 7 randomly selected ears. The number of kernels per ear was calculated by multiplying the number of kernel rows by the number of kernels per row, while the number of kernels per square meter (KSQ) was calculated by multiplying the number of kernels per ear by the number of ears per square meter (Testa et al., 2016). All the collected ears were shelled using an electric ear sheller and the grain moisture content was determined using a Dickey-John GAC100 grain analyzer (Auburn, IL, USA). Grain samples were dried in a forced-air oven at 60°C until constant weight to express the yield as dry matter and for the subsequent analyses regarding the qualitative and sanitary traits. A sub-sample of 200 kernels was weighed to obtain the thousand kernels weight (TKW). The test weight (TW) was determined on dry kernels, by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp).

5.3.8. Kernel quality and sanitary traits

The grain quality traits were assessed through the kernel test weight (TW), as calculated on dry kernels using a Dickey-John GAC100 grain analyzer (Auburn, IL, USA) and the grain protein content (GPC), which was determined according to AACC (2000).

The sanitary traits were assessed from the ECB severity, which was calculated as the percentage of kernels per ear characterized by injuries due to ECB larva activity, while ear rot severity was calculated as the percentage of kernels per ear with symptoms, according to Blandino et al. (2009a). The ECB and ear rot severity were both evaluated on a sub-sample of 20 ears. Furthermore, fumonisin B_1 and B_2 (FBs) and deoxynivalenol (DON) contaminations were analyzed using the ELISA method, by means of direct competitive immunoassays (RIDASCREEN® Fumonisin and RIDASCREEN® DON, R-Biopharm, Darmstadt, Germany), according to the manufacturer's instructions. Briefly, both mycotoxins were extracted for 15 min by mechanically shaking, at 100 rpm, (shaker model M102-OS, MOM Instruments, Milan, Italy) 20 g samples with 100 ml of methanol/distilled water, (70:30, v/v) for FBs and with distilled water for DON, respectively. After extraction and filtration through Whatman® n.1 299 filters, 50 µl of diluted filtrate was used for the ELISA test. The optical density was measured at 450 nm using an ELISA 96-well plate reader (Das srl, Rome, Italy), and all the standard and sample solutions were analyzed in duplicate wells. The limit of quantification (LOQ) of the analytical method was set at 250 µg kg^{-1} and 37 µg kg^{-1} for FBs and DON, respectively.

5.3.9. Data analysis

Data were analyzed using a mixed effects model where the experimental site, tillage technique and starter fertilization strategy were considered as fixed effects, while the maize hybrid and the year - nested effects, - were considered as random factors. Graphical analysis of the residuals was used to assess the homoscedasticity and normality of the data and when not normally distributed, data were log-transformed and normality checked again (Onofri et al., 2016). A logarithmic transformation was applied to the FBs and DON values. As the DON contamination at the Carmagnola site was smaller than the LOQ of the instrument for both experimental years, the mixed model was only applied at the Poirino site, considering the tillage technique and the starter fertilization strategy as fixed effects and the maize hybrid and the year - nested effects - as random factors. When single factors or their interaction determined significant effect, the means were compared using the Bonferroni post hoc test at $p \leq 0.05$. Regarding interactions site × tillage and site × starter fertilization, means referred to different tillage or starter fertilization were separated within each site. The statistical procedure was performed using the lmer function in the LME4 statistical package of the R software (R Core Team, 2019).

5.4. Results

5.4.1. Weather and soil conditions

The two growing seasons showed slightly different meteorological trends for both rainfall and temperature (expressed as GDDs in Table 2). June 2019 had less rainfall than June 2020, which was the wettest month of the 2020 growing season. The wettest month in 2019 was July and was concurrent with the maize anthesis stage. The 2019 growing season was characterized by cooler temperatures during April and May, while June was warmer, thus resulting in similar cumulative GDDs for the two growing seasons at both experimental sites.

The soil temperature at sowing differed between the two tillage techniques by ~1 °C in either years or sites. The highest values were found in the plowed soils and ranged from 9°C (Poirino-2020) to 12°C (Carmagnola-2019). The soil temperatures measured during the seedling stages are reported in Figure 1 and are expressed as GDDs. The soil temperatures confirmed the air temperature trends of the two experimental years. If we consider the plowed soil as a reference, the GDDs reached at 42 DAS were 140°C and 100°C higher in 2020 than in 2019 at Carmagnola and Poirino, respectively. The PLOW treatment showed higher temperatures than the ST treatment at both locations, but with differences between the two soils; the greatest differences between treatments were observed at Poirino. For example, in 2019, the differences in GDDs at 42 DAS between the PLOW and ST were 17°C and 44°C at Carmagnola and Poirino, respectively, while they were 34°C and 54°C, respectively, in 2020.

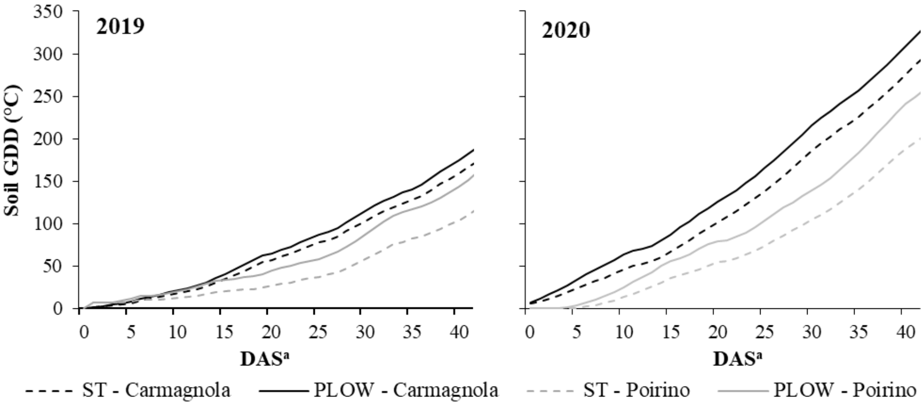


Figure 1. Cumulative soil growing degree days (10°C base; GDDs, at a depth of 7 cm) over the first 42 days after sowing (DAS) of the maize crop cycle in the 2019 and 2020 growing seasons at Carmagnola and Poirino for the plowing (PLOW) and strip-tillage (ST) systems.

5.4.2. Plant emergence and early crop vigor

The tillage technique for seedbed preparation and the experimental site clearly affected the plant emergence pattern, while the starter fertilization strategy did not (Table 4). The number of emerged plants was lower in the ST treatment than in the PLOW one for both measurement times. Moreover, in correspondence to the “50% emergence” measurement, the comparison of the density of emerged plants between the two tillage techniques showed more pronounced differences for Poirino (p <0.001; Figure S1), where the PLOW treatment had 4.4 plants m^{-2} and the ST treatment had 1.0 plants m^{-2}, while the crop density for Carmagnola was 5.5 and 3.3 plants m^{-2} for the PLOW and ST treatments, respectively. The tillage technique and the starter fertilization strategy at sowing clearly affected the maize plant development, as assessed by means of the NDVI measurements during the vegetative stages (Figure 2). The NDVI values were summarized by the AUCDC index, thus allowing the differences in plant development during the early growing stages (Table 4) to be assessed. Generally, the PLOW treatment resulted in a significant (p <0.001) increase in the AUCDC, compared to the ST system, with a significantly more marked effect at Poirino (+20%) than at

Carmagnola (+7%) (Figure 3a). On the other hand, the DAP treatment performed better than DIG and the NT control. The starter fertilization strategy differed between the two soil types (*p* <0.001), and between the two tillage techniques (*p* <0.001). In fact, the DIG treatment at Carmagnola showed an intermediate AUCDC value between the DAP and NT treatments (26.1 *vs* 29.0 and 24.8), while no differences were recorded at Poirino, compared to DAP (Figure 4a). The crop growth determined by the DIG injection at sowing did not show any differences from the NT when the soil was plowed, while it was higher than NT (+12%) when the seedbed was prepared by ST (Figure 5).

The results regarding the plant height during the vegetative growth stages (GS 15 and GS 33) confirmed those of the AUCDC index (Table 4). The plant height in the PLOW treatment increased significantly (*p* <0.001) by 34% and 56% at GS 15 and GS 33, respectively, compared to the ST. The starter fertilization with DAP recorded the highest plant height for both measurement times, followed by the DIG and the NT treatments. However, an interaction with the site was observed, with a more pronounced positive effect of PLOW than of ST at Poirino (+222%) than at Carmagnola (+12%), at GS 33 (Figures 3b and 3c). Another factor that affected the plant height was the starter fertilization strategy, which again showed a different pattern at the two sites, both at the GS 15 and GS 33 growing stages. During steam elongation, the plant height in the DIG treatment was 35% higher than in NT, and 28% lower than in DAP at Carmagnola, while it was higher than NT (+229%) but similar to DAP at Poirino (Figure 4b and 4c), thus enhancing the differences recorded during leaf emission.

The differences due to the tillage technique and to the starter fertilization strategy recorded during vegetative development were confirmed at flowering and harvest (Table 4). On average, maize anthesis in the PLOW system occurred 2.2 days before the ST system. However, this reduction in days from sowing to plant flowering was significantly (*p* <0.001; Figure S2) higher at Poirino (-3.4 days) than at Carmagnola (-1.1 days). The starter fertilization clearly affected the flowering date. When DIG was supplied, flowering occurred at 94.4 DAS, while it occurred at 92.4 and 97.0 DAS in the DAP and NT treatments. The earlier flowering resulted in a grain moisture content reduction at harvest. On average, the PLOW technique showed a decrease of 1.2% in the grain moisture content, compared to the ST system, while the DAP treatment resulted 0.8% drier than DIG, which was 1.1% drier than the NT treatment. Furthermore, the three-way interaction was significant. The DIG treatment showed a similar grain moisture content (24.6%) to the NT treatment (25.2%) for both tillage techniques at Carmagnola, while the DIG treatment at Poirino did not differ from the DAP treatment (Figure S3).

Table 4. Effect of site, tillage and starter fertilization on maize emergence, early plant vigor, expressed as the plant height at the leaf emission (GS15) and stem elongation stages (GS33), the area under the canopy development curve (AUCDC), the date of flowering, expressed as days after sowing (DAS), and grain moisture content at harvest for 2019 and 2020.

Factor	**Source of Variation**	**50% Emergence (plants m^{-2})**	**100% Emergence (plants m^{-2})**	**Plant height GS 15 (cm)**	**Plant height GS 33 (cm)**	**AUCDC**	**Date of flowering (DAS)**	**Grain moisture (%)**
Site (Si)[a]	Carmagnola	4.4 a	7.2 b	12.7 b	50.4 a	26.6	87.5 b	24.4 b
	Poirino	2.7 b	7.6 a	14.0 a	26.8 b	26.9	101.7 a	25.6 a
	p(F)	*< 0.001*	*0.048*	*0.045*	*< 0.001*	*n.s.*	*< 0.001*	*< 0.001*
Tillage (T)[b]	ST	2.2 b	7.2 b	11.4 b	30.2 b	25.1 b	95.7 a	25.6 a
	PLOW	4.9 a	7.6 a	15.3 a	47.0 a	28.4 a	93.5 b	24.4 b
	p(F)	*< 0.001*	*0.002*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Starter fertilization (SF)[c]	DIG	3.6	7.6	12.9 b	42.4 b	26.7 b	94.4 b	24.9 b
	DAP	3.6	7.2	15.8 a	50 a	28.5 a	92.4 c	24.1 c
	NT	3.4	7.3	11.3 c	23.5 c	25.1 c	97.0 a	26.0 a
	p(F)	*n.s.*	*n.s.*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Si × T	*p(F)*	*< 0.001*	*n.s.*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Si × SF	*p(F)*	*n.s.*	*n.s.*	*< 0.001*	*< 0.001*	*0.001*	*n.s.*	*n.s.*
T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*0.001*	*n.s.*	*n.s.*
Si × T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*0.027*

[a] The site values are based on 48 observations (2 years ×2 tillage systems × 3 starter fertilizations × 4 repetitions).

[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[c] The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Means followed by different letters are significantly different at p <0.05

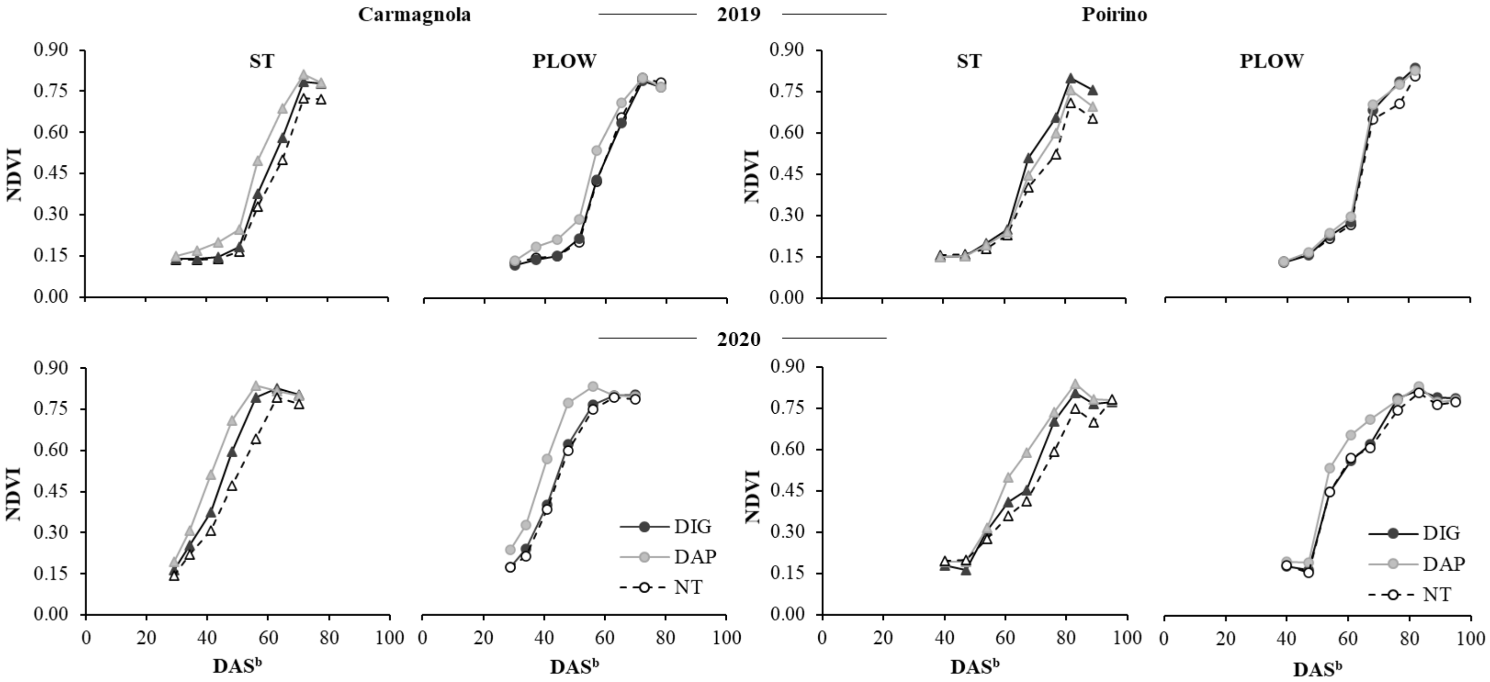


Figure 2. Effect of the starter fertilization strategies on the maize canopy development, expressed as NDVI from the 3-leaf stage to tasseling within the two compared tillage systems: strip-tillage (ST; triangle symbols) and plowing (PLOW; circle symbols) at Carmagnola (left side) and Poirino (right side) experimental sites on the 2019 (upper row) and 2020 (lower row) growing seasons.

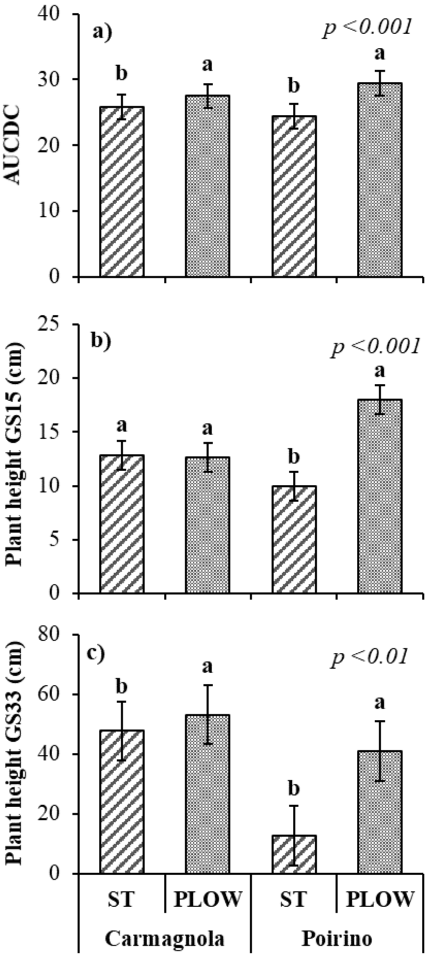


Figure 3. Effect of the tillage system, at each experimental site, on the early vigor of maize expressed as the area under the canopy development curve (AUCDC; a), the plant height at the leaf emission (GS15; b) and the stem elongation stages (GS33; c) for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Means with different letters within each site are significantly different. Error bars show the ± standard error.

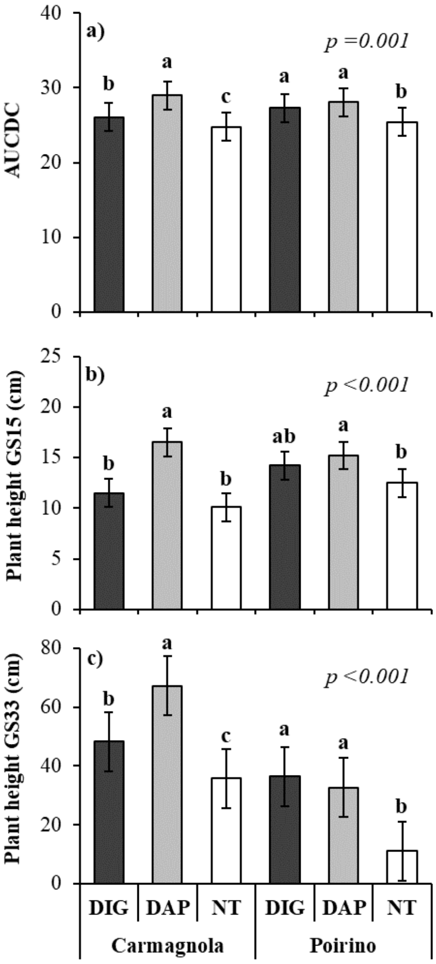


Figure 4. Effect of the starter fertilization at each experimental site on the early vigor of maize expressed as the area under the canopy development curve (AUCDC; a), the plant height at the leaf emission (GS15; b) and the stem elongation stages (GS33; c) for field experiments carried out in the 2019 and 2020 growing seasons. Means with different letters within each site are significantly different. Error bars show the ± standard error.

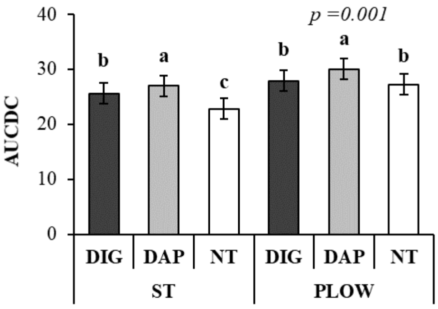


Figure 5. Effect of the starter fertilization within each tillage system on the early vigor of maize expressed as the area under canopy development curve (AUCDC) for field experiments carried out in the 2019 and 2020 growing seasons. Means with different letters are significantly different. Error bars show the ± standard error.

5.4.3. Plant morphological traits

The final crop density was affected by the experimental site and the tillage technique, but not by the fertilization strategy (Table S2). The crop density was higher at Poirino (7.9 plants m^{-2}) than at Carmagnola (7.4 plants m^{-2}). The tillage technique effect confirmed what was observed at emergence, i.e. PLOW resulted in 0.3 plants m^{-2} more than ST. The ear density exhibited the same trend as the crop density, while no effects of the analyzed factors were recorded on the percentage of barren plants.

The stalk area, the plant height at maturity and the ear insertion height reported in Table S2 are proxies of plant stability. The ST system led to an increase in stalk area, compared to the PLOW system, whereas the DAP and DIG similarly increased the stalk area in comparison to the NT treatment. The lowest plant and ear insertion height at maturity was recorded when DAP was supplied at sowing.

5.4.4. Grain yield and yield components

The grain yield was affected by the site and the starter fertilization strategy, while no effects were recorded for the tillage technique as a single factor (Table 5). The highest yield was registered at Carmagnola (16.4 Mg ha^{-1}), where 6% more was produced than at Poirino. The DAP and the DIG applications at sowing led to a similar significant grain yield increase, compared to the NT treatment (+11 and +12%, respectively). The significant interaction site × tillage indicated that the grain yield was decreased by the ST adoption of 0.9 Mg ha^{-1} at Poirino. On the contrary, ST recorded higher grain yield (+1 Mg ha^{-1}) than PLOW at Carmagnola (Figure S4).

The significant three-way interaction showed the same ear density regardless the tillage technique and the starter fertilization at Carmagnola site, while the ST treatment recorded lower values than PLOW at Poirino site (Figure S5). Although, the ear length was 1.5% higher for the ST treatment than for the PLOW one and the number of rows was 2.5% higher for PLOW than for ST, no differences were observed for the number of kernels per row (Table S3) or for the number of kernels per ear as a result of the tillage technique (Table 5). In contrast, the number of kernels per ear was differently affected by the starter fertilization in the two sites. Compared to the NT treatment, DAP increased the number of kernels per ear by 11% when applied to plowed soil, while recorded intermediate values and not significantly different when applied to the strip-tilled soil. When DIG was deep-injected, either in plowed or strip-tilled soils, yielded a similar number of kernels per ear of DAP in plowed soils (Figure S6). Owing to the higher crop density observed in the PLOW treatment, KSM was also enhanced by 6%, compared to ST (Table S3), although TKW was not affected by the tillage system as single factor (Table 5). However, the significant interaction site × tillage (p =0.039; Figure S7a) showed that TKW in the ST system was higher (+2.4%) than in the PLOW system at the Carmagnola site. In contrast, the tillage system did not affect the TKW at Poirino.

All the analyzed yield components, except TKW, were significantly affected by the starter fertilization. The DAP fertilization determined an increase in the cob length (+2.5%), compared to NT, while the number of rows per ear decreased (-2.4%), compared to DIG. Furthermore, the DAP and DIG treatments led to a similar increase in the number of kernels per row, the number of kernels per ear and the KSM to the untreated control. The starter fertilization only affected the

TKW at Carmagnola (*p* <0.001; Figure S7b), where DAP and DIG were characterized by similar kernels weights (+4 and +6% than NT, respectively).

Table 5. Effect of site, tillage and starter fertilization on maize grain yield and yield components: ear density, number of kernels (K) per ear and thousand kernels weight (TKW) for 2019 and 2020. DM: dry matter.

Factor	**Source of Variation**	**Grain yield ($t\ ha^{-1}$ DM)**	**Ear density ($ear\ m^{-2}$)**	**K ear^{-1}**	**TKW (g)**
Site (Si)[a]	Carmagnola	16.4 a	7.2 b	623 a	381 a
	Poirino	15.5 b	7.7 a	594 b	362 b
	p(F)	*0.013*	*0.006*	*< 0.001*	*< 0.001*
Tillage (T)[b]	ST	15.9	7.3 b	603	373
	PLOW	15.9	7.6 a	614	371
	p(F)	*n.s.*	*0.016*	*n.s.*	*n.s.*
Starter fertilization (SF)[c]	DIG	16.6 a	7.6	619 a	374
	DAP	16.4 a	7.4	624 a	375
	NT	14.8 b	7.4	582 b	366
	p(F)	*< 0.001*	*n.s.*	*0,008*	*n.s.*
Si × T	*p(F)*	*< 0.001*	*n.s.*	*0.008*	*0.039*
Si × SF	*p(F)*	*n.s.*	*0.048*	*n.s.*	*0.002*
T × SF	*p(F)*	*n.s.*	*n.s.*	*0.044*	*n.s.*
Si × T × SF	*p(F)*	*n.s.*	*0.034*	*n.s.*	*n.s.*

[a] The site values are based on 48 observations (2 years ×2 tillage systems × 3 starter fertilizations × 4 repetitions).

[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[c] The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Means followed by different letters are significantly different at $p < 0.05$

5.4.5. Grain qualitative and sanitary traits

The qualitative parameters of the grain were affected by the experimental site and the starter fertilization, while the tillage system did not show any significant effects (Table 6). The highest grain hardness value, expressed as TW, was recorded at Poirino (78.1 kg hl^{-1}). The starter fertilization performed with DAP and DIG equally increased TW (+1.2%), compared to NT. On the other hand, GPC was higher at Carmagnola, while the starter fertilization improved GPC when DIG was compared with NT, while DAP was in between.

Only the location affected the ECB and ear rot severities (p <0.001 and p =0.002, respectively; Table 6). Since all the experiments were treated with foliar insecticides, the injury caused by the ECB activity was generally low, with less damage observed at Poirino than at Carmagnola. Ear rot severity was significantly higher in Poirino than in the Carmagnola site, while tillage and starter fertilization did not affect the ear surface with mold symptoms at harvest at either site. The FBs contamination was higher (p =0.001) at Carmagnola than at Poirino, while DON was only detected in Poirino (Table 7). Although the starter fertilization did not affect ECB or the ear rot severity, the DIG injection on average lowered FBs contamination (p =0.002). However, differences were found between the two experimental sites: DIG and DAP behaved similarly in Carmagnola, while the DIG treatment in Poirino recorded the lowest FBs contamination, while DAP showed an intermediate FBs contamination compared to the NT and DIG treatments (Figure 6). The grain DON contamination in Poirino was negatively affected by the adoption of ST, which increased it by 118%, compared to PLOW, although no significant effect of starter fertilization was observed.

Table 6. Effect of site, tillage and starter fertilization on qualitative and sanitary traits, expressed as kernels test weight (TW), grain protein content (GPC), European Corn Borer (ECB) injuries and ear rot severity on maize ears at harvest for 2019 and 2020.

Factor	**Source of Variation**	**TW (kg hl^{-1})**	**GPC (%)**	**ECB severity (%)**	**Ear rot severity (%)**
Site (Si)[a]	Carmagnola	76.5 b	9.3 a	3.7 a	2.3 b
	Poirino	78.1 a	8.9 b	2.1 b	6.1 a
	p(F)	*< 0.001*	*0.004*	*< 0.001*	*0.002*
Tillage (T)[b]	ST	77.2	9.2	2.6	4.4
	PLOW	77.5	9.0	3.1	4.7
	p(F)	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Starter fertilization (SF)[c]	DIG	77.6 a	9.3 a	2.9	4.2
	DAP	77.6 a	9.1 ab	3.0	5.1
	NT	76.7 b	8.9 b	2.8	4.3
	p(F)	*0.002*	*< 0.001*	*n.s.*	*n.s.*
Si × T	*p(F)*	*n.s.*	*0.020*	*n.s.*	*n.s.*
Si × SF	*p(F)*	*n.s.*	*< 0.001*	*n.s.*	*n.s.*
T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Si × T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

[a] The site values are based on 48 observations (2 years ×2 tillage systems × 3 starter fertilizations × 4 repetitions).

[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[c] The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Means followed by different letters are significantly different at $p < 0.05$.

Table 7. Effect of tillage and starter fertilization on fumonisins B_1 + B_2 (FBs) and deoxynivalenol (DON) contaminations for 2019 and 2020.

Factor	**Source of Variation**	**FBs**		**DON**	
		T	**N (µg kg^{-1})**	**T**	**N (µg kg^{-1})**
Site (Si)[a]	Carmagnola	7.21 a	4699	-	< 37
	Poirino	6.49 b	3998	-	188
	p(F)	*0.001*		-	
Tillage (T)[b]	ST	6.89	4457	5.11 a	275
	PLOW	6.81	4267	4.35 b	101
	p(F)	*n.s.*		*0.002*	
Starter fertilization (SF)[c]	DIG	6.47 b	3449	4.57	114
	DAP	6.97 a	5209	4.55	150
	NT	7.12 a	4466	5.07	301
	p(F)	*0.002*		*n.s.*	
Si × T	*p(F)*	*n.s.*		-	
Si × SF	*p(F)*	*< 0.001*		-	
T × SF	*p(F)*	*n.s.*		*n.s.*	
Si × T × SF	*p(F)*	*n.s.*		-	

The FBs and DON contamination means reported are transformed [T; y' = ln (x + 1)] and non-transformed (N) values. The DON content in the Carmagnola site was always below the Limit of Quantification (LOQ) of the ELISA analysis, 37 µg kg-1. The reported data only refer to the Poirino experiment in 2019 and 2020.

[a] The site values are based on 48 observations (2 years ×2 tillage systems × 3 starter fertilizations × 4 repetitions).

[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[c] The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Means followed by different letters are significantly different at $p < 0.05$.

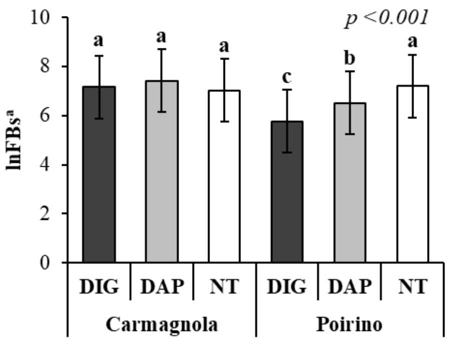


Figure 6. Effect of the starter fertilization at each experimental site on fumonisins $B_1 + B_2$ *(FBs) contamination for field experiments carried out in the 2019 and 2020 growing seasons. Means with different letters within each site are significantly different. Error bars show the ± standard error.*

[a] The reported FBs contamination means are transformed $[y' = \ln(x + 1)]$.

5.5. Discussion

The adoption of the two tested tillage techniques and starter fertilization with deep-injected DIG or sub-surface placed DAP, did not show on average any differences in terms of grain yield. However, important differences were recorded for the early plant development.

5.5.1. Effect of the tillage technique

The ST technique determined a significant delay in the early crop growth, probably because it hampered the soil heat from building-up during the emergence and seedling stages, compared to PLOW. The reduction in soil temperatures with ST could be due to the presence of crop residues on the soil surface in the no-tilled areas (Griffith et al., 1973; Gauer et al., 1982). The overall effect of tillage techniques on maize production depends to a great extent on their effect on the initial crop establishment, starting from crop emergence (Trevini et al., 2013). Cooler soil temperatures (< 12.5°C), combined with a higher soil water content during the emergence period, increase the time required for seedling emergence, decrease the emergence rate due to longer seed exposure to soil pathogens, and determine a limited root surface area that could reduce the nutrient uptakes (Cutforth et al., 1986; Imran et al., 2013), in particular in soils with a low drainage capacity (Dwyer et al., 2000). The soil at the Poirino site, which is characterized by a slightly finer texture and lower drainage capacity than that of the Carmagnola site, showed a limited heat build-up that slowed down the germination, emergence and early growth of maize for both tillage techniques, but in particular when ST was adopted. The Carmagnola soil, with a coarser texture and better drainage, showed higher soil temperatures and a general faster emergence and early growth of maize, but also limited differences between the strip-tilled and the plowed treatments.

Despite the delay in early development of ST, the final yield in the considered growing seasons was similar for the two tillage systems. The absence of an overall effect of tillage on yield could have been masked by the favorable late summer weather recorded for both growing seasons, which may have allowed complete maturation, even for strip tillage.

Among the yield components, tillage influenced mainly the crop density and also cob length, and caused a smaller number of KSM in the ST treatment. These findings agree with the study of Rashidi and Keshavarzpour (2007), who recorded a lower crop density for a variety of reduced tillage techniques. The same authors also reported a reduced cob length and a higher number of grain rows per ear for minimum tillage, while our study showed opposite trends, like those of Ali et al. (2012).

Among the yield quality traits, GPC and TW were similar in the two tillage systems, while the grain moisture content at harvest was different. The grain moisture was higher by 1.2% for the ST treatment, thus implying a delay in grain moisture dry down, compared to PLOW. This was probably due to the fact that anthesis was postponed by 2.2 days in ST, as also reported by Hudon et al. (1990).

The content of mycotoxin in ST could be higher as a consequence of both a higher inoculum production (crop debris on soil surface) and higher infection and disease development (due to a later flowering and ripening). Tillage could affect the grain sanitary traits because of both later flowering and ripening or the different incorporations of the previous crop residues into the soil. The late development of maize cultivated in ST, with a late shift of ripening, could result in a higher severity of ECB in the absence of a direct control of this insect (Blandino et al., 2009a) or condition more favorable for fungal development, thus leading to a possible higher risk of mycotoxin contamination.

As far as the role of debris is concerned, previous studies conducted on wheat and other small cereals (Blandino et al., 2010) reported remarkable effects of tillage on *Fusarium* head blight infection and the DON contamination of wheat. Debris is the primary reservoir of *Fusarium* inoculum (Koch et al., 2006), and DON contamination in wheat is more severe if the preceding crops is maize, particularly when minimum tillage or no-tillage is applied (Blandino et al., 2012). Conversely, only limited information has been reported for the role of previous crop management on soil tillage in maize (Palumbo et al., 2020). The present work, carried out by limiting the contamination linked to ECB injuries through a direct control of the vectors, has highlighted that the occurrence of debris on the soil surface with ST did not increase the risk of contamination by FBs, the most frequently detected mycotoxins in temperate maize growing areas. Although the present experiment was carried out in growing seasons with a low DON contamination, the ST treatment resulted in a significant increase in this mycotoxin, compared to PLOW. This finding confirms the hypothesis advanced by Munkvold (2014) who suggested that the tillage technique of maize has a greater influence on the fungi that produce DON (e.g., *F. graminearum* or *F. culmorum*, which are characterized by an inoculum splash dispersal), than on FBs-producing fungi (e.g. *F. verticillioides* or *F. proliferatum*). In fact, although crop residues are also the most important source of inoculum for FBs-producing fungi, these species produce large numbers of aerially dispersed microconidia, and the effects of debris burying, as a result of plowing, could be negligible in areas with high prevalence of maize, because of their long-distance air dispersal (Munkvold, 2014).

In order to limit the occurrence of DON in maize, as a consequence of the additive effect of the agronomic factors involved in the cropping system, it would be preferable not to apply ST in environments in which there is cool and prolonged ripening, or where late maturity hybrids, high plant populations or nitrogen fertilization are adopted (Blandino et al., 2009b).

5.5.2. Effect of the starter fertilization

The organic or mineral NP starter fertilization allowed the early development gap between the ST and PLOW treatments to be compensated for, compared to NT (Fig. 5). As extensively reported in literature, our results confirm the synergistic effect of combined applications of N and P on early crop vigor, as assessed by the plant height and the AUCDC index growing curves.

The type of starter fertilization (i.e. DIG or DAP), which supplied equal amounts of P at sowing but different nutrient forms and placement and timing of N application, influenced significantly early maize development, as shown by the plant height and AUCDC index. The DIG treatment equaled the crop development of DAP at Poirino, while it caused a slower growth rate at Carmagnola, thus indicating an important effect of the type of soil, also regarding nutrient availability. The Carmagnola soil had a lower concentration of plant available-P and sub-alkaline pH, which may favor P sorption (Barrow, 2017), than the Poirino soil. Consequently, the more readily available mineral form of the sub-surface placed DAP, although with a small N rate compared to that applied through DIG, was better utilized by small plants than the organic form of deep-injected DIG, which required a mineralization process and could have caused an additional local increase in soil pH due to its intrinsic alkalinity (Tampio et al., 2016). This suggested that supplying soluble P fertilizers in bands near the seed in situations where soil P reactions are dominated by sorption may improve the nutrient use efficiency (Battisti et al., submitted). An early availability of N and P provides an additional advantage to cereals, as it determines the potential grain number for physiological reasons (Angus et al., 2019).

The differences observed between the sub-surface placed DAP and the deep-injected DIG band, in terms of early crop growth enhancement, could also be linked to the depth of the fertilizer placement, that is, 5 cm apart from the seed in the case of DAP and ~20 cm below the seed in the case of DIG. These distances were chosen to avoid possible toxic effects of the fertilizer on the seedling, as young roots may encounter reducing conditions, high concentrations of ammonium and a high pH, which may cause plant death (Sawyer and Hoeft, 1990; Salvagiotti et al., 2013), when manure is placed too close to the seeds. The tested distances resulted in no negative effects on seed germination or the plant density, compared to NT. This agreed with the results of Pedersen et al. (2020b), who suggested a distance of at least 5 cm between the manure band and the seed row to avoid root damage following slurry injection. However, nutrients placed in a deeper position as DIG band may be less plant available during early stages of plant growth when root density is still low at high depths (Nkebiwe et al., 2016). Nonetheless, the digestate deep-injection improved the early maize vigor compared the NT treatment where no N and P were applied at sowing, indicating that a fraction of these nutrients became available for plant uptake concurrently with the plant requirement. Slurry or biogas digestate deep band placement into the root zone in one operation with tillage represents a suitable technique to improve N efficiency of organic fertilizers in ST system. Indeed, due to the occurrence of previous crop debris on soil surface, the top-dressed urea distribution in ST is applied without any incorporation into the soil, thus increasing losses by ammonia volatilization (Souza et al., 2016). Otherwise, band injection can reduce the temporary immobilization of manure N because of the smaller interface between manure and soil, as well as ammonia volatilization (Schröder et a., 2015; Federolf et al., 2016). Further investigations should be conducted to optimize the placement distance of digestate for the tested pedo-climatic situations. Further investigations should be conducted to optimize the placement distance of digestate for the tested pedo-climatic situations.

The advantage, in terms of early development and earlier flowering of starter NP fertilization, led to a significant increase in grain yield and test weight. Although the type of fertilization, deep-injected DIG or sub-surface placed DAP, influenced the early growth of maize at sowing, it did not affect the yield or most of the grain quality traits. The only quality parameter that was influenced by the type of fertilization was the grain protein content, which was significantly increased when digestate was used. This was probably because only part of the total applied N in digestate is immediately available, while the organic fraction is slowly released through the mineralization process over the growing season, thus leading to an improved synchrony between N demand and N availability (Silva et al. 2005). In the environment where this study was conducted, the application of mineral NP fertilizer as a starter fertilization, especially when combined with an early sowing date, could result in an overall lower mycotoxin contamination, since maize flowering and kernel development occur for weather conditions that are less favorable for fungal infection and, for FBs, the ripening can partially skip the attack of ECB (Blandino et al., 2022). In the present study, in which a foliar insecticide was applied to minimize the role of injuries caused by the insect vector that favors mycotoxin contamination, a starter fertilization with both deep-injected DIG and sub-surface placed DAP was also effective in reducing the FBs contamination at Poirino. This effect is probably related to the earlier flowering and the earlier maturity of the fertilized treatments than the control, although a role could also be played by the observed increase in ear length and grain yield, which contribute to diluting the proportion of ears affected by ear rot (Blandino et al., 2008).

5.6. Conclusion

This study has provided useful information on how tillage techniques and starter fertilization strategies, based on the placement of N and P in bands at specific distances from seed rows, affect maize emergence, early plant growth, yield components, as well as qualitative and sanitary grain traits. Our findings highlighted the suitability of the adoption of the ST technique in different soils, especially when conjugated with a proper starter fertilization management performed with digestate or DAP. The deep injection of digestate showed moderate advantages on the early development of maize and allowed the same yield to be achieved as the mineral starter fertilization. Furthermore, digestate injection, together with strip tillage, enables farmers to prepare their seedbeds and distribute manure in a single operation, thus saving time and reducing fuel consumption. In addition, ammonia emissions after manure spreading are reduced after an injection, compared to a broadcast application, and the farm N use efficiency is therefore increased (Federolf et al, 2016).

The combination of these two good practices can therefore increase the environmental and economic sustainability of farms, without significant impairments of the production level, grain quality or sanitary aspects, compared to more conventional techniques that involve the use of mineral fertilizers and plowing.

This study focused on the first-year effects of both practices. Future research should address the medium and long-term effects, and also explore a wider annual variability, as several effects on grain traits could be magnified over years with adverse ripening conditions.

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5.7. Supplementary information

Table S1. Fertilization management of each treatment at each experimental site.

Site	**Starter fertilization**[a]	**N (kg ha^{-1})**		**P_2O_5 (kg ha^{-1})**
		At sowing	**Top-dressing**	**At sowing**
Carmagnola	**DIG**	202	75	69
	DAP	27	250	69
	NT	0	250	0
Poirino	**DIG**	202	0	69
	DAP	27	175	69
	NT	0	175	0

[a] DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Table S2. Effect of site, tillage and starter fertilization on crop density and plant stability, expressed as the stalk area, plant height at maturity and ear insertion height for 2019 and 2020.

Factor	**Source of Variation**	**Plant density (plant m^{-2})**	**Stalk area (cm^2)**	**Plant height at maturity (cm)**	**Ear insertion height (cm)**
Site (Si)[a]	Carmagnola	7.4 b	5.4 a	337 a	165 a
	Poirino	7.9 a	4.6 b	295 b	148 b
	p(F)	*0.002*	*< 0.001*	*< 0.001*	*< 0.001*
Tillage (T)[b]	ST	7.5 b	5.1 a	317	156
	PLOW	7.8 a	4.9 b	316	157
	p(F)	*0033*	*0.022*	*n.s.*	*n.s.*
Starter fertilization (SF)[c]	DIG	7.8	5.3 a	320 a	161 a
	DAP	7.6	5.0 a	310 b	152 b
	NT	7.6	4.7 b	318 a	157 a
	p(F)	*n.s.*	*< 0.001*	*0.001*	*< 0.001*
Si × T	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Si × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Si × T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

[a] The site values are based on 48 observations (2 years ×2 tillage systems × 3 starter fertilizations × 4 repetitions).

[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[c] The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Means followed by different letters are significantly different at $p < 0.05$

Table S3. Effect of site, tillage and starter fertilization on maize yield components: cob length, number of rows per ear, number of kernels (K) per row and number of kernels per square meter (KSM) for 2019 and 2020.

Factor	**Source of Variation**	**Cob length (cm)**	**Row ear^{-1} (n)**	**K row^{-1}**	**KSM**
Site (Si)[a]	Carmagnola	21.3 a	16.6	37.6 a	4468
	Poirino	19.8 b	16.4	36.4 b	4591
	p(F)	*< 0.001*	*n.s.*	*0.049*	*n.s.*
Tillage (T)[b]	ST	20.7 a	16.3 b	37.1	4401 b
	PLOW	20.4 b	16.7 a	37.0	4657 a
	p(F)	*0.047*	*0.016*	*n.s.*	*0.004*
Starter fertilization (SF)[c]	DIG	20.7 ab	16.7 a	37.2 a	4670 a
	DAP	20.8 a	16.3 b	38.2 a	4608 a
	NT	20.3 b	16.4 ab	35.7 b	4309 b
	p(F)	*0.008*	*0.045*	*< 0.001*	*0.002*
Si × T	*p(F)*	*n.s.*	*n.s.*	*0.028*	*n.s.*
Si × SF	*p(F)*	*n.s.*	*0.043*	*n.s.*	*n.s.*
T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Si × T × SF	*p(F)*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

[a] The site values are based on 48 observations (2 years ×2 tillage systems × 3 starter fertilizations × 4 repetitions).

[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[c] The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

Means followed by different letters are significantly different at $p < 0.05$

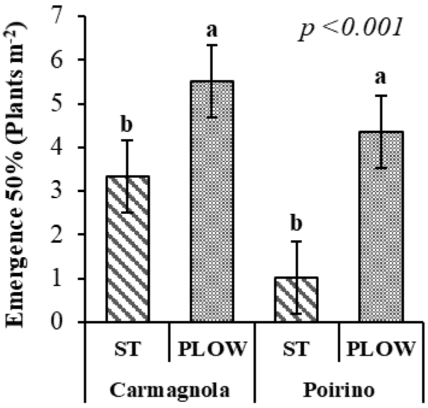

Figure S1. Effect of the tillage system, at each experimental site, on the maize emergence for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Means with different letters within each site are significantly different. Error bars show the ± standard error.

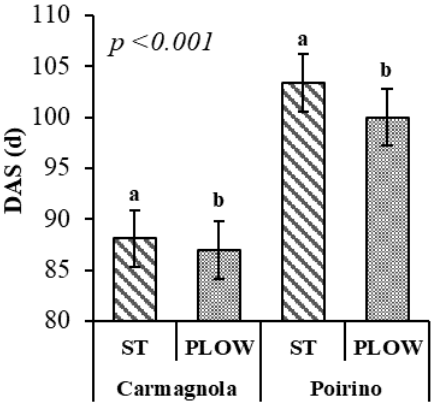

Figure S2. Effect of the tillage system, at each experimental site, on the date of flowering expressed as days after sowing (DAS) for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Means with different letters within each site are significantly different. Error bars show the ± standard error.

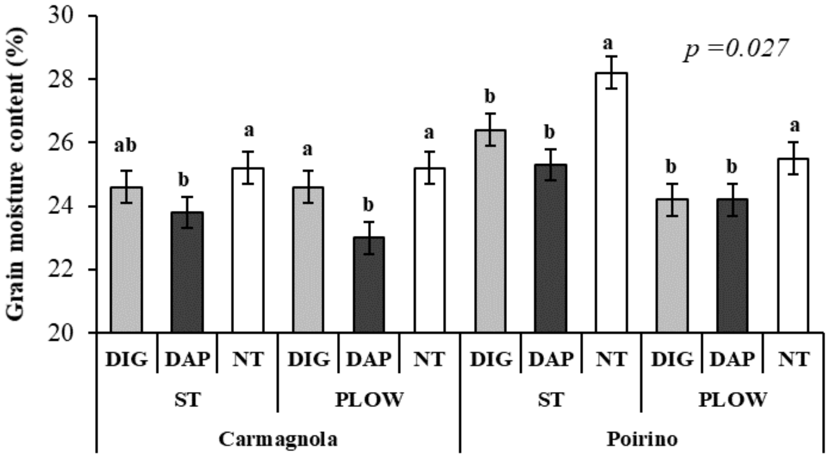

Figure S3. Effect of the starter fertilization within each tillage system and each experimental site on the grain moisture content for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing. Means with different letters are significantly different. Error bars show the ± standard error.

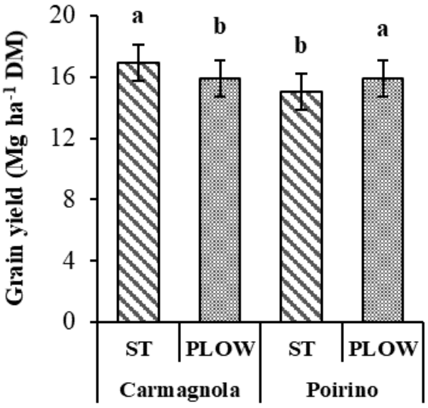

Figure S4. Effect of tillage system on grain yield, express as dry matter (DM), for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Means with different letters within each site are significantly different. Error bars show the ± standard error.

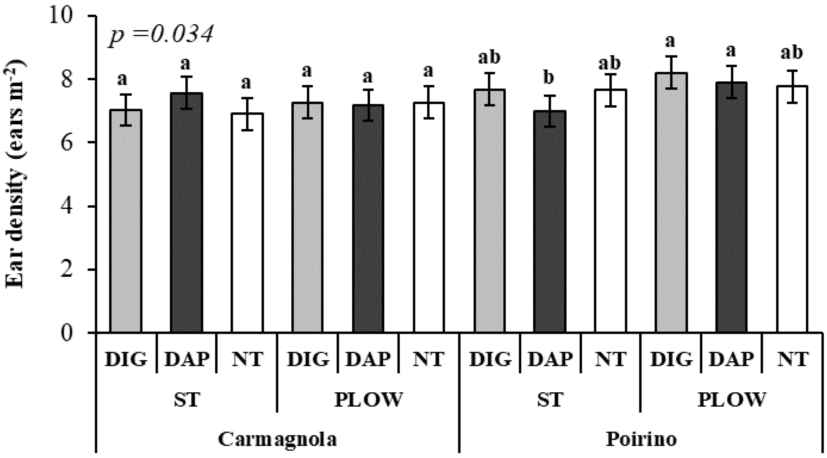

Figure S5. Effect of the starter fertilization within each tillage system and each experimental site on the ear density for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing. Means with different letters are significantly different. Error bars show the ± standard error.

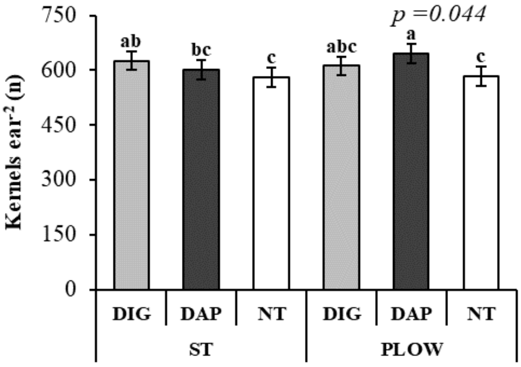

Figure S6. Effect of the starter fertilization and tillage system on the number of kernels per ear for field experiments carried out in the 2019 and 2020 growing seasons. Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing. Means with different letters are significantly different. Error bars show the ± standard error.

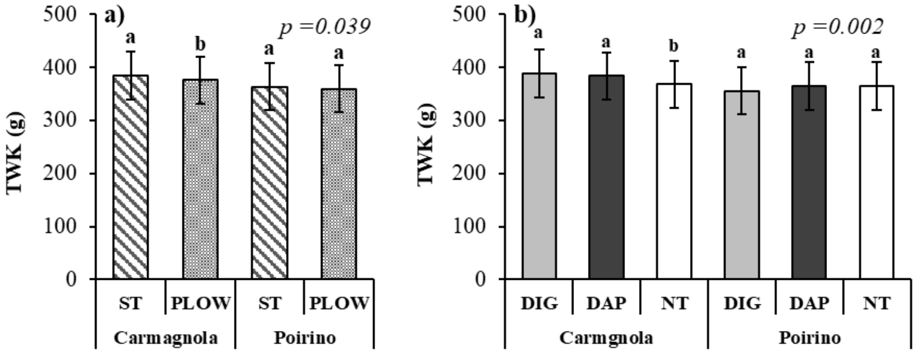

Figure S7. Effect of tillage system (a) and the starter fertilization (b) at each experimental site on the thousand kernels weight (TWK) for field experiments carried out in the 2019 and 2020 growing seasons. Soil tillage system: ST, strip tillage; PLOW, plowing. Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing. Means with different letters are significantly different. Error bars show the ± standard error.

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6. The phosphorus fertiliser value of sewage sludge ash for pasture production on soils that differ in phosphate buffering and phosphate sorption capacity

Authors: Michela Battisti[1,2], Richard J. Simpson[1], Adam Stefanski[1], Alan E. Richardson[1], Rebecca E. Haling[1*]

Affiliation:
[1] CSIRO Agriculture and Food, GPO Box 1700, Canberra ACT 2601, Australia
[2] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, Grugliasco (TO), 10095, Italy

Corresponding author: Rebecca E. Haling, CSIRO Agriculture and Food, GPO Box 1700, Canberra ACT 2601, Australia.
E-mail: Rebecca.Haling@csiro.au

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6.1. Abstract

The global phosphate rock reserves that underpin phosphorus (P) fertiliser production are finite. Recovery of P from waste streams (e.g., by producing fertiliser products from sewage sludge) is an important way to conserve scarce P resources.

This study evaluated the P fertiliser and soil pH amendment values of a municipal sewage sludge ash (SA) for pasture growth on three acidic soils with contrasting P buffering and P sorption ("fixation") characteristics. Growth of subterranean clover (*Trifolium subterraneum* L.) in pots and incubation of the fertilisers with soil were used to compare the initial (following application) and residual (following incubation with soil) fertiliser values of SA with those of single superphosphate (SSP).

Soil pH_{Ca} was increased linearly by application of SA. The lime-equivalent value of SA was ~33% (product weight basis). The initial P-fertiliser value of SSP was substantially greater than that of SA in all three soils. For both P sources, initial fertiliser value was negatively related with the P buffering capacity of the soil. The residual P fertiliser value of SSP ranged from low (42% of initial fertiliser value) to relatively high (82%) indicating differences among the soils in their P sorption capacities. The residual value of SA was better than that of SSP in soils that exhibited strong P-sorption capacities. Standard soil-P tests (i.e., Colwell P, $CaCl_2$-P, Bray-1 P) that are often used in combination with SSP, underpredicted the fertiliser value of SA. This indicated a need to recalibrate these soil tests for use with SA.

Keywords: Incinerated sewage sludge ash; phosphorus recycling; slow-release phosphorus; phosphorus soil tests; circular P economy

6.2. Introduction

Phosphorus (P) is one of the key fertiliser nutrients that are essential for global food security (Stewart et al., 2005; Sutton et al., 2013; Sattari et al., 2014). Most mineral P fertiliser is manufactured from phosphate rock, a non-renewable resource (Ulrich and Frossard, 2014). The extraction, manufacture and use of fertiliser P is marked by substantial inefficiencies in handling and use (e.g., Weaver and Wong, 2011; Scholz and Wellmer, 2015, 2018; Lun et al., 2018) and it is generally agreed that it is important to close the anthropogenic P cycle by reducing these inefficiencies and by maximising opportunities for recycling P (Stamm et al., 2021).

A high proportion of the P in agricultural produce is ultimately channelled into the waste streams of cities (e.g., Kalmykova et al., 2012; Schoumans et al., 2015). Sewage, in particular, is an important interception point for recycling this P back to agriculture. More than 30 million Mg of dry sewage sludge are produced annually in developed countries alone (Le Blanc et al., 2008) and the value of recycled P from sewage as a replacement for mined P is potentially significant. For example, it is estimated that recovering waste stream P in Europe could replace 17-31% of mineral P fertiliser by 2030 (Huygens et al., 2019; Tonini et al., 2019). In subsistence and smallholder farming systems, where poor access and high fertiliser costs restrict the use of P fertilisers, recycling waste stream P may even eliminate the P deficits in some farm production systems (e.g., Krause and Rotter, 2018). However, for Australian agriculture which predominantly services export markets and has a relatively high P requirement due to the widespread existence of P deficient soils that also accumulate (“fix”) P after it is applied (Weaver and Wong, 2011; Simpson et al. 2011a; Simpson et al., 2015), the replacement value of recycling P is lower. Recycling every unit of domestic waste stream P could theoretically only supply ~9% of the fertiliser P required (Cordell et al., 2013).

There are a number of issues that impact the use of sewage sludge as a P fertiliser. It is a bulky product which adds considerably to handling and spreading costs, P concentrations can be low, variable and with poor bioavailability depending on the source and temporal influences (range: 2 to 30 g P kg^{-1} after primary digestion treatment; Pathak et al., 2009; Mateo-Sagasta et al., 2015), sewage sludge may be contaminated with heavy metals, xenobiotic pollutants, microbial and viral pathogens, and is associated with offensive odours (e.g., Pathak et al., 2009; Fijalkowski et al., 2017; Case et al., 2017). In some European countries, these issues have ultimately resulted in bans on the spreading of sewage sludge on farms (Schoumans et al., 2015).

Incinerating sewage sludge addresses some of these issues as the incineration process eliminates odour, organic and microbial contaminants, and reduces some of the bulk associated with sludge. In processes where the sludge has been treated with lime (EPA, 2000), SA delivers a useful pH amendment co-benefit for very acid soils (Dann et al., 1989; Leech et al., 2019). In some instances, the liming benefit is considered to be of equivalent or greater value than the P fertiliser value of SA (Dann et al., 1989).

The total P concentration of sewage sludge ash (SA; e.g., ~60-120 g P kg^{-1}; Kruger and Adam, 2015) can be comparable to that of mineral P fertiliser (e.g., 90 g P kg $superphosphate^{-1}$). However, the bioavailability of the P is often low (e.g., only ~10-46% of P content soluble in ammonium citrate extractant). This reduces its utility as a fertiliser (Nanzer et al., 2014; Kruger and Adam, 2015). Furthermore, the availability of P from SA application may be modified by soil pH (Cabeza et al., 2011) and may be influenced by the choice of crop (Vogel et al., 2015). Research is showing that sewage sludge incineration methods can be modified to deliver future SA products with substantially improved P bioavailability and reduced heavy metal content (Herzel et al., 2016). However, large quantities of SA are presently used as mine-fill, in construction materials, or disposed of in landfill (Kruger and Adam, 2015; Donatello and Cheeseman, 2013) and P is effectively being lost from use in agricultural systems.

There are a number agronomic issues that need to be addressed to support consumer demand for SA if it is to be an effective substitute for soluble-P fertilisers. Agronomic research demonstrates repeatedly that “traditional” soluble-P fertilisers deliver larger crop and pasture yields per unit P applied than less bio-available P products (Sale et al., 1997; Lewis et al., 1997; Joseph et al., 2019; Leech et al., 2019). Guidelines exist for the use of soluble-P fertilisers across different soil types (Gourley et al., 2019), and they are used by farmers with confidence (e.g., Simpson et al., 2009). There are numerous studies that demonstrate SA applications can also improve plant growth on acid, low-P soils e.g., Dann et al., 1989; Cabeza et al., 2011; Vogel et al., 2015; Leech et al., 2019; Raymond et al., 2019). However, the magnitude of yield responses to SA is always lower in the year of application than that achieved using a soluble-P fertiliser. It is an equal problem that the effectiveness of SA varies substantially among soils within a local district (Leech et al., 2019). An understanding of why this occurs and how to calculate SA fertiliser rates for different soils, is needed to ensure they can be used as substitutes for, or to complement traditional P fertilisers.

Longer term studies also indicate improving release of P from SA with time. For example, after a single, large application of SA (2.5 t ha^{-1}; 6.6% total P with 1.1% as citrate-soluble P), it was observed that pasture growth at three field sites was relatively poor in the year of application, but improved progressively over a 5-year period. Pasture yield in year 5 was ~3 to ~7-fold that achieved in the year of application. The range in residual effectiveness varied among the field sites. Other studies report similar results, some also showing that yield increases were accompanied by increased P concentrations in herbage (Jakobsen and Willett, 1986; Mackay et al., 2017; Joseph et al., 2019). This change in residual value of SA as a P fertiliser and why this varies among soils is unclear.

In this paper, we report on soil incubations and plant growth experiments to assess the P fertiliser and soil pH amendment values of a SA soil-amendment product produced by Australia’s largest inland by incineration of municipal sewage and wastewater treatment plant. The fertiliser value of SA was compared with that of P supplied as superphosphate which is the dominant soluble-P fertiliser used for pasture production in Australia. Our hypotheses were: (i) that soil P-buffering (adsorption) reactions were likely to modulate the effectiveness of SA applications when applied fresh to a soil, as these reactions were known to modify the amount of soluble P fertiliser that must be applied to increase soil P fertility (Burkitt et al., 2001), and (ii) that the slow P-sorption reactions that ultimately lead to the accumulation of P in a soil (sometimes also referred to as "P-fixation"; Barrow, 1999) would determine the residual fertiliser value of SA as it does for soluble-P fertilisers (Barrow, 1974; Bolland, 1986). Pasture growth responses were, therefore, compared among three acid soils that were anticipated to have contrasting P-buffering and P-sorption attributes. The ability of standard extractable soil-P tests to detect changes in P fertility after application of SA was also examined to understand how to measure soil fertility when SA was used as an alternative P fertiliser.

6.3. Materials and methods

6.3.1. Fertilisers

Two sources of P were compared: (i) Single superphosphate (Superfect®, Incitec Pivot Pty Ltd, Southbank, Victoria, Australia; hereafter referred to as SSP) which contained 97 g P kg^{-1}, of which 77 g P kg^{-1} was water-soluble P and 86.4 g P kg^{-1} was citrate-soluble (Table 1).

Table 1. Main chemical characteristics of the single superphosphate (SSP) and incinerated sewage sludge ash (SA) products compared in the experiments.

	Unit	SSP	SA
Total P[a]	g kg^{-1}	97.4	79.8
Water soluble P[b]	g kg^{-1}	76.8	0.0
Citrate soluble P[c]	g kg^{-1}	86.4	48.5
Sulfur[d]	g kg^{-1}	120	9.0
Fe[a]	g kg^{-1}	4.5	102.0
Al[a]	g kg^{-1}	4.3	7.6
pH[e]		2.9	12.2

Chemical analysis of the alternative products used in the present experiments were undertaken utilising the 'FA-PACK 005 Fertiliser Soluble Phosphorus' analysis service offered by the Environmental Analysis Laboratory, Southern Cross University, Lismore, NSW, Australia.

[a] sample digested with HNO_3 and HCl and measured by inductively coupled plasma-mass spectrometry (ICP-MS); method 3125, American Public Health Association (2018)

[b] extracted by shaking fertiliser in deionised water (1:100 w/v) for 1 h at 22°C

[c] fertiliser shaken in an aqueous 20 mg citric acid L^{-1} solution (1:100 w/v) for 1 h at 22°C

[d] sample digested with HNO_3 and HCl and measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES); method 3125 from American Public Health Association (2018)

[e] measured after shaking fertiliser in deionised water (1:100 w/v)

SSP is the most widely used P fertiliser for pastures in Australia. It also contains an appreciable amount of sulfur (120 g S kg^{-1}) as sulphate and had a pH in water of 2.9. The SSP was ground finely and sieved to <710 µm prior to use. (ii) Incinerated sewage sludge ash (Agri-Ash®, Icon Water Ltd, Canberra, ACT, Australia; hereafter referred to as SA) which contained 80 g P kg^{-1} (nil as water-soluble P; 48.5 g as citrate-soluble P kg^{-1} and 9 g S kg^{-1}, Table 1). The pH in water of SA was 12.2. Agri-Ash is produced by the thermal treatment (750-1000°C) of municipal primary and secondary sewage sludge at the Lower Molonglo Water

Quality Control Centre (Icon Water Ltd). Sewage ash is alkaline because lime is added as part of the sewage treatment process. Addition of lime also assists the precipitation of P. The final product is distributed as a soil amendment in fine powder form (<600 µm).

6.3.2. Soils

For the present study three soils of low available-P status were collected from permanent pastures in southern New South Wales, Australia.

(i) A sandy loam soil, hereafter referred to as the ‘Wallaroo’ soil after the farm location from which it was sourced, was collected from the top 20-150 mm depth of a Yellow Chromosol soil profile (Isbell, 1996) at the Ginninderra Experiment Station, Canberra, ACT (35°10′30” S, 149°02′33.4″ E).

(ii) A loamy clay soil, hereafter referred to as the ‘Kia-Ora’ soil, was collected from the topmost 20-150 mm depth of a Yellow Kurosol soil profile (Isbell, 1996) in the Bookham district of NSW (34°48′6.18” S, 148°34′49.17″ E).

(iii) A clay loam soil, hereafter referred to as the ‘Merrill’ soil, was collected from the topmost 20-150 mm depth of a Red Kurosol soil profile (Isbell, 1996) in the Gunning district of NSW (34°38'40.86" S, 149°19'49.90" E).

The soils were collected in October 2019, sieved (<2 mm) to remove any rocks and root material and steam pasteurised at 65ºC for 2 hours prior to their use in all plant growth experiments. This treatment suppresses oomycete and fungal root pathogens if present in the soil. In a previous study, George et al. (2005) found that the pasteurisation process did not significantly alter total P or Colwell P concentrations, soil pH, extractable cation concentrations or organic matter content in five different Australian soils. Key chemical properties of the soils after steam pasteurisation are reported in Table 2. They indicated that the three soils differed in their P-adsorption capacities (as measured by the Phosphorus

Buffering Index [PBI] test; Burkitt et al., 2002; 2008), the soils were acidic, potentially Al-toxic for root growth, and potentially deficient in S and P for clover growth (Gourley et al., 2019).

Table 2. Chemical characteristics of the soils.

Parameter	Unit	Wallaroo	Kia-Ora	Merrill
pH$_{Ca}$[a]		4.5	4.4	4.0
Conductivity[b]	dS m^{-1}	0.096	0.155	0.055
Organic Carbon[c]	%	0.86	1.51	3.55
Colwell P[d]	mg kg^{-1}	7	8	18
PBI[e]		40	72	151
Colwell K[f]	mg kg^{-1}	302	154	329
KCl40-Sulfur[g]	mg kg^{-1}	4.3	3.4	4.4
N present as NH_4^+[h]	mg kg^{-1}	18	23	29
N present as NO_3^-[h]	mg kg^{-1}	52	79	8
Exchangeable Al[i]	meq 100 g^{-1}	0.38	0.68	2.25
Exchangeable Ca[i]	meq 100 g^{-1}	1.64	1.32	1.9
Exchangeable Mg[i]	meq 100 g^{-1}	0.76	1.03	0.82
Exchangeable K[i]	meq 100 g^{-1}	0.48	0.21	0.68
Exchangeable Na[i]	meq 100 g^{-1}	0.05	0.16	0.07
Al (%CEC)	%	11.5	20.0	39.3

[a] pH 1:5 *w/v* 0.01 M $CaCl_2$ (Rayment and Lyons, 2011; Method 4B4)
[b] Electrical conductivity of 1:5 *w/v* H_2O (Rayment and Lyons, 2011; Method 3A1)
[c] Wet oxidation (H_2SO_4 is added to soil wetted with a $Cr_2O_7^{2-}$ solution) (Rayment and Lyons, 2011; Method 6A1)
[d] Bicarbonate-extractable P (Colwell, 1963) (Rayment and Lyons, 2011; Method 9B)
[e] Phosphorus Buffering Index (Rayment and Lyons, 2011; Method 9I2c)
[f] Bicarbonate-extractable K (Rayment and Lyons, 2011; Method 18A1)
[g] Potassium Chloride – 40 Sulfur (Rayment and Lyons, 2011; Method 10D1)
[h] Mineral-N – 2 M KCl (Rayment and Lyons, 2011; Method 7C2b)
[i] Exchangeable bases, CEC and anion exchange capacity by compulsive exchange, no pre-treatment for soluble salts (Rayment and Lyons, 2011; Method 15E1)

Preliminary experiments were conducted to further characterise: (i) the pH buffering capacity of each soil (i.e., the amount of lime required to increase pH$_{Ca}$ by one unit) using unpasteurised soil, (ii) the potential for Al toxicity, and (iii) the extent of P, S and any other nutrient limitations for growth of *Trifolium subterraneaum* L. (subterranean clover) in each of the soils after pasteurisation.

6.3.3. Preliminary soil characterisation experiments

Soil pH buffering and liming-value of the sewage ash: Six rates of lime (0, 0.5, 1.0, 2.0, 4.0 and 5.0 mg kg soil^{-1}) and six rates of SA (0.065, 1.25, 2.5, 5.0, 10.0 and 15.0 mg kg soil^{-1}) were mixed thoroughly into each of the three soils in plastic bags. The finely ground lime (F70 superfine limestone, 380 g Ca kg^{-1} and 10 mg Mg kg^{-1} as carbonate; Omya Australia P/L, Lindfield) had a high neutralising value (97%) to ensure rapid reactivity. The soils were then watered to a predetermined weight to achieve 70% of their drained upper limit (field capacity). Soil moisture was maintained at this level throughout the test period by regular weighing and rewatering. Subsamples (~40 g of moist-soil) were taken from each treatment after 4, 7 and 32 days and were dried at 40 °C. Soil pH (1:5 *w/v*; 0.01 M $CaCl_2$) was measured and the amount of lime required to raise soil pH by one unit was determined for each of the three soils (pH buffering), as was the lime-equivalent value of SA when it was applied to these soils.

Al-toxicity bio-assay: Four plants differing in their Al tolerance were grown in each of the three soils to determine whether the soils were toxic for root growth. Two near-isogenic genotypes of *Triticum aestivum* L. (wheat) that differ at a single locus for Al-induced malate export from root tips: ES-8 (an acid-sensitive genotype) and ET-8 (an acid-tolerant genotype) (Delhaize et al., 1993), *Medicago littoralis* Rhode cv. Herald (strand medic, an acid-sensitive annual pasture legume), and *T. subterraneum* cv. Leura (subterranean clover) were grown in square pots (55 × 55 × 80 mm height) containing 0.24 kg oven dry equivalent of soil with 6 plants per pot. The native pH_{Ca} of the three soils ranged from 4.0-4.5 and Al (% CEC) ranged from 12-39% (Table 2). Control treatments were created by incorporating lime at a rate appropriated for each soil (Table 3) to achieve pH_{Ca} 5.5. The treatments were replicated three times (Wallaroo and Kia-Ora soils) or twice for the Merrill soil. Plants were grown in a controlled-environment growth chamber with 12 h of light (720 µmol quanta^{-1} m^{2} s^{-1}) and 12 h of dark, at 20 and 15 °C, respectively. Pots were arranged in a randomised complete block design. Soil moisture was maintained at 80% of field capacity by daily watering to a predetermined weight. Plants were harvested four days after seedling emergence. The root system was washed free of soil and the length of the main axis root was measured using a ruler graduated in millimetres.

Subtractive nutrient experiment: All macro- and micronutrients likely to be deficient for plant growth in these soils were applied to each of the three soils to create "all-nutrient" controls (Table S1). An additional all-nutrient plus lime treatment was created by also applying the appropriate amount of lime to achieve pH_{Ca} 5.5. Subtracted nutrient treatments were created by omitting individually: P, S, K, or all of the micronutrients (Table S1). The subtractive experiment was conducted at the native low pH of each soil because it was known from the Al-bioassay that Al levels would not be toxic for growth of *T. subterraneum*. However, a small amount of lime was mixed into the Merrill soil to adjust its pH_{Ca} to 4.2 because germination of clover seeds was inhibited in the unamended Merrill soil at pH_{Ca} 4.0.

Cylindrical polyvinyl chloride (PVC) pots (87 mm internal diameter, 200 mm height) were filled with 0.9 kg (for Kia-Ora and Wallaroo) or 0.75 kg oven-dry basis (for Merrill) of unamended soil (i.e., no fertiliser added) to form a subsoil layer. A fertilised topsoil (0.30 kg of soil; oven-dry basis) consisting of each of the subtractive nutrient treatments was added on top of the unamended subsoil layer. This arrangement mimics the stratification of nutrients that occurs naturally in field soils, and especially after the application of fertiliser to the soil surface of grazing lands. The plant growth system was developed to reflect growth in pasture swards and to permit studies of root morphology. It is known to reasonably reflect the response of legume pastures to nutrient management in the field. A more extensive description of the merits of the plant growth system is provided by Becquer et al. (2021). Nine plants of *T. subterraneum* cultivar Leura were established per pot. *Trifolium subterraneaum* was used as the test species in this and subsequent experiments because it is the most widely grown pasture legume in southern Australia. The plants were grown in a controlled-environment growth cabinet with conditions as per the bioassay. Pots were arranged in a randomised complete block design with 4 replicates. The position of each pot within each block was randomly changed weekly to minimise any positional effects within the growth cabinet. Sleeves with a reflective inner surface were fitted to the outside of the pots and raised daily to equal plant height to mimic the light condition in a pasture sward. Soil moisture was maintained at 80% of field capacity by daily watering to a predetermined weight and once per week each pot was watered to field capacity to avoid subsoil drying. Seven days after planting the clover was inoculated with Group C *Rhizobium* (strain WSM 1325, NewEdge Microbials, Albury, NSW, Australia). Plants were harvested six weeks after sowing when still in the vegetative growth stage. Shoots were cut at the soil surface and rinsed with water to remove any soil and were subsequently dried at 70 °C to determine the dry mass.

Extractable P and soil pH responses to product application and incubation

One kilogram (oven-dry basis) subsamples of each soil (sieved <2 mm; unpasteurised) were mixed thoroughly in polythene bags with either SSP (at rates of 0.909, 1.82 or 3.41 g kg soil^{-1}), or SA (at rates of 2.5, 5.0, or 10.0 g kg soil^{-1}). An unfertilised treatment was included as a control. Water was added to each soil sample to achieve 70% of field capacity. Each treatment was replicated three times. The bags were incubated for ~6 months at 25°C. Soil moisture was maintained at 70% of field capacity by periodic weighing and watering. Subsamples of the treated soils (40 g of moist soil) were taken from each bag after 0, 7, 35, 62, 91 and 172 days of incubation and were dried at 40°C prior to analysis for Colwell P (method 9B2 in Rayment and Lyons 2011) and pH (1:5 *w/v*; 0.01 M $CaCl_2$).

Plant growth responses to the soils and fertilisers

Microswards of *T. subterraneum* cv. Leura (9 plants pot^{-1}) were grown in each of the three soils and shoots were harvested 5 weeks after sowing as described previously (see: Subtractive nutrient experiment). For each soil treatment, the pots were initially filled with a subsoil layer of the soil to which basal nutrients, except P, had been applied (0.75 kg oven dry basis for Merrill; 0.9 kg soil for Kia-Ora and Wallaroo). A small number of alkathene beads were placed around the edge of each pot to mark the upper face of the subsoil before a topsoil layer of the soil was added (0.3 kg oven dry basis).

Each topsoil layer contained basal nutrients and lime and an appropriate amount of a P-fertiliser treatment. The P treatments were created by applying six rates of either SSP (0.114, 0.227, 0.455, 0.909, 1.82 and 3.41 g kg topsoil^{-1}) or SA (0.625, 1.25, 2.5, 5.0, 10.0 and 15.0 g kg topsoil^{-1}) into the topsoil layer of each pot. The fertilisers were either applied fresh, or had been previously applied and then incubated with the soil for 98 days at 25°C and at a constant moisture content (70% field capacity). All nutrients were mixed uniformly through the topsoil layer. A control with no P fertiliser was also included. There were four replicates of the Wallaroo and Kia-Ora soil treatments, and three replicates of the Merrill soil treatments.

The basal nutrients included: 41 mg kg dry soil^{-1} $MgSO_4 \cdot 7H_2O$, 43 mg kg^{-1} $CaSO_4 \cdot 2H_2O$, 169 mg kg^{-1} KNO_3, 28 mg kg^{-1} $(NH_4)_2SO_4$, 17 mg kg^{-1} NH_4NO_3, 119 µg kg^{-1} H_3BO_3, 759 µg kg^{-1} $MnCl_2 \cdot 4H_2O$, 359 µg kg^{-1} $ZnSO_4 \cdot 7H_2O$, 33 µg kg^{-1} $CuSO_4 \cdot 5H_2O$, 72 µg kg^{-1} $(NH_4)_2MoO_4$, 20 µg kg^{-1} $CoCl_2 \cdot 6H_2O$ and 1530 µg kg^{-1} Fe-EDTA. Their application ensured that the availability of all nutrients, except P, would not be limiting for plant growth. Lime was applied to each topsoil layer to achieve pH_{Ca} 5.5 in each soil and for treatments with SA, the lime rate was modified to account for the liming value of the SA. In most cases, the initial topsoil pH that was actually achieved was marginally above pH 5.5 and at high rates of SA the topsoil pH was well above this (Wallaroo: pH_{Ca} 6.7-7.4; Kia-Ora:

pH_{Ca} 6.7-6.9) despite no lime having been added to these treatments (Table S2; Fig. S1).

Initial and residual values of the P fertilisers

The initial P-fertiliser value of each product was determined as the gradient of the plant growth response to freshly applied product (shoot yield per unit P applied). Soil for the freshly applied treatments was pasteurised prior to adding each product.

When P is applied to many soils it continues to react slowly with the soil (Barrow, 1980a, b; Sample et al., 1980; Barrow, 1999) causing plant-available phosphate to become only sparingly-available for plant uptake (McLaughlin et al., 2011). The effectiveness of P fertiliser applications is, consequently, reduced over the time. In the present experiment, the fertilisers were incubated with each soil at 25°C to mimic the continuing, “slow reaction” of phosphate with soil that typically occurs over a longer timeframe in the field (Barrow, 1974). The residual value of each fertiliser in each of the three soils was then assessed by expressing the gradient of the plant growth response in the incubated soil, as a proportion of the gradient of growth response to a fresh application of the fertiliser (Barrow, 1974; Bolland, 1986). The residual value of SSP (i.e., essentially a water-soluble phosphate source) was used, subsequently, as a measure of the P-sorption capacity of each soil. Soil for the incubated treatment was pasteurised after the incubation period.

Harvesting soil and roots: final soil chemical measurements (all treatments) and roots harvests (Wallaroo-soil treatments only), were achieved by removing the soil from the pots as an intact core. Each soil core was cut with sharp knives using a series of jigs to ensure accurate cuts. Initially, the cores were cut at the interface of the fertilised topsoil (0-45 mm depth) and the subsoil (45-190 mm depth) as identified by the alkathene beads. Then each topsoil section was cut vertically into two quarter-segments and a half-segment. One quarter-segment of the soil was dried at 40°C for determination of soil pH (1:5 *w/v*; 0.01 M $CaCl_2$) and Colwell P. The other quarter was not used. The roots in the half-segment were washed free of soil over a 1 mm sieve and root dry mass was determined after they had been dried at 70°C. The subsoil was divided vertically into two equal sections. The subsoil roots from one half-segment were harvested and used to determine root mass as per the topsoil roots.

To determine shoot and root P concentrations, dried tissue samples were ground or chopped to a fine powder. Tissue samples of 20 or 50 mg were reduced to ash in a muffle furnace for 4 h at 550 °C. The ash was dissolved in 2M HCl (1 mL acid per 10 mg of tissue) and P concentration of samples was determined colorimetrically at 630 nm using malachite green (Irving and McLaughlin, 1990). The P content of the shoots, topsoil and subsoils roots were summed to calculate total plant P uptake per pot.

Root mass fractions were calculated separately as the mass of roots in the topsoil, subsoil or total root system divided by the total plant mass. The relative shoot yield was calculated as shoot yield at the given level of P per pot divided by the maximum potential shoot yield.

6.3.4. Soil test P methods

At the beginning of the main P fertilizer response experiment (i.e. P fertilizer × P rate experiment), the extractable P concentrations of each topsoil treatment were determined using four soil test methods with differing extraction chemistries: (i) Colwell P in which P was extracted using 0.5 M $NaHCO_3$ adjusted to pH 8.5 with NaOH, a soil/solution ratio of 1:100, and an extraction time of 16 h at 25°C (method 9B2, Rayment and Lyons, 2011); (ii) $CaCl_2$-extractable P, which is considered to reflect the soil solution P concentration, with an extraction for 18 h at 25°C by 0.005 M $CaCl_2$ at a 1:5 soil/solution ratio (method 9F2, Rayment and Lyons, 2011); (iii) Bray-1 P in which P was extracted by a dilute solution of 0.03 M NH_4F and 0.025 M HCl at a 1:7 soil/solution ratio for an extraction time of 60 seconds at 25°C (method 9E2, Rayment and Lyons, 2011) and (iv) BSES P, that was initially developed to predict the P responsiveness of soils used for sugar cane in Queensland, required the extraction of a soil/solution ratio of 1:200 with 0.005 M H_2SO_4 for 16 h at 25°C (method 9G2, Rayment and Lyons, 2011).

6.3.5. Data analysis

Data from the bioassay, subtractive and main pot experiments were analysed separately for each soil using either a one- or two-way analysis of variance depending upon the number of factors. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. A 5% level of significance was applied for all statistical tests. Statistical analysis was performed using R software version 3.6.3 (R Core Team, 2020).

The critical rates of total P applied as SSP or SA (i.e., the rate of application required to achieve 90% of maximum shoot yield; g P kg soil^{-1}) and their 95% confidence intervals were estimated for each fertiliser treatment using the Dyson and Conyers (2013) yield response function in Excel 2019 (Microsoft Corporation). Maximum (asymptotic) shoot yield was defined as the average of the shoot dry mass recorded when 1.82 and 3.41 g SSP kg soil^{-1} was applied to the Wallaroo soil (Fig. 1a); this did not differ from the highest yields achieved in the Kia-Ora and Merrill soils when SSP was applied at rate of 3.41 g kg soil^{-1} ($P > 0.05$).

The initial fertiliser value (i.e., effectiveness of freshly applied fertiliser) of each product was determined as the gradient of linear regressions fitted to the data for shoot dry matter response to total P applied in the region of each response function before shoot yields departed from linearity. The residual value of each product was calculated as the gradient of the regression fitted to each incubated treatment divided by the gradient of the response in the freshly applied treatment (Barrow, 1974; Bolland, 1986).

6.4. Results

6.4.1. Soil characteristics and nutrient requirements

Soil pH buffering and liming-value of the sewage ash: The Merrill soil had the highest soil pH buffering capacity and required 4.55 g lime kg soil^{-1} to raise the soil pH by 1 unit. The equivalent lime requirements of the Wallaroo and Kia-Ora soils were 1.60 and 1.56 g kg soil^{-1}, respectively (Table 3). The average lime-equivalent value of SA was 33.1% of the value of applying lime (product dry weight basis; range 29.7 to 35.0 among the three soils).

Table 3. pH buffering by the three soils and the relative liming-value of sewage ash (SA).

Soil	pH_{Ca} response to lime or sewage ash incorporation[a]			pH buffering[b]	Relative liming value of the sewage ash[c] (%)
Wallaroo	Lime:	$y = 0.60x + 4.29$	$R^2 = 0.96$	1.60 ±0.08	34.6 ±3.3
	SA:	$y = 0.21x + 4.31$	$R^2 = 0.96$		
Kia-Ora	Lime:	$y = 0.63x + 4.08$	$R^2 = 0.99$	1.56 ±0.04	29.7 ±1.4
	SA:	$y = 0.19x + 4.17$	$R^2 = 0.99$		
Merrill	Lime:	$y = 0.21x + 3.96$	$R^2 = 0.98$	4.55 ±0.18	35.0 ±2.6
	SA:	$y = 0.08x + 3.98$	$R^2 = 0.98$		
Average relative liming value					33.1 ±2.4

[a] $y = pH_{Ca}$; x = rate of incorporated product (g kg soil^{-1})
[b] Rate of lime application (g kg soil^{-1}) required to raise the pH_{Ca} by 1 unit
[c] Relative liming value of the sewage ash (%) calculated as the ratio of pH change per unit weight of applied lime and pH change per unit weight of sewage ash applied

Al-toxicity bio-assay: In the absence of lime amendment, the Merrill soil had a high concentration of extractable Al (39.3% of CEC; Table 2). Root elongation of *T. aestivum* var. ES-8 and M. *littoralis* cv. Herald in the unamended soil was 85 and 51%, respectively, of that in the lime-amended soil (Table 4; Fig. S2) and *T. subterraneum* failed to germinate in the Merrill soil without lime amendment. However, there was no significant effect on the root elongation of *T. aestivum* var. ET-8 in the unlimed Merrill soil. The Al concentration of the unamended Kia-Ora soil was relatively high (20.0% of CEC) and root elongation of ES-8 and cv. Herald was significantly inhibited (87 and 81%, respectively) relative to root length in the lime-amended soil. Germination by subterranean clover was not affected in the unlimed Kia-Ora soil and the minor reduction in root elongation rate was not statistically significant. The Wallaroo soil had a comparatively low Al concentration (11.5% of CEC) and root length of the four genotypes was not significantly affected.

Table 4. Key characteristics of the soils determined by bioassay.

Lime and nutrient requirements (subtracted nutrient experiment)				
Nutrient treatment	*Unit*	*Wallaroo*	*Kia-Ora*	*Merrill*
All+lime	Shoot yield achieved as % of All nutrient control treatment	101	76	83
-P		19	24	20
-K		96	94	96
-S		102	92	97
-micronutrients		100	100	99
Nil		18	25	23

Al-toxicity (root growth bioassay)				
Test genotype	*Unit*	*Wallaroo*	*Kia-Ora*	*Merrill*
Wheat var. ET8 (Al-tolerant)	Length of main root axis achieved in unlimed soil (pH_{Ca} 4-4.5; Table 1) as % of root length achieved in limed soil (pH_{Ca} 5.5)	99	97	97
Subterranean Clover cv. Leura (Al-tolerant)		86	91	0
Wheat var. ES8 (Al-tolerant)		97	87	85
Strand medic cv. Herald (Al-sensitive)		94	81	51

Subtractive nutrient experiment: In each of the three soils, maximum shoot dry mass of *T. subterraneum* (2.1-2.2 g pot^{-1}) was achieved in the "all nutrients" treatment (Fig. S3 and Table 3) and this did not differ from the shoot yield of plants grown without addition of K, S or micronutrients. However, the All+lime treatment achieved only 76% and 83% of the shoot dry mass in the All-nutrient treatment in the Kia-Ora and Merrill soils, respectively. Lime incorporation did not reduce shoot yield in the Wallaroo soil. Shoot yields in the minus P treatment were very low in all three soils (19%, 24% and 20% of the yield achieved in the All-nutrient treatment in Wallaroo, Kia-Ora and Merrill soils, respectively) and were not different to the yields recorded in the Nil treatments. Despite the low KCl40-S soil test results for all of the soils (3.4-4.4 mg S kg^{-1}, Table 2) indicating potential for S deficiency (Gourley et al., 2019), the subtractive nutrient experiment demonstrated that only P was limiting for growth of *T. subterraneum*.

6.4.2. The extractable P and soil pH responses to the fertiliser application and soil incubation

The initial Colwell P concentration of all three soils was increased linearly ($P < 0.05$; $R^2=1.00$) by incorporating increased amounts of SSP. The rates of change in extractable P concentration were 583, 613 and 451 mg Colwell P per g P applied in the Wallaroo, Kia-Ora and Merrill soils, respectively (derived from Fig. S4). At the highest rate of SSP incorporation, incubation of the SSP-fertilised Wallaroo soil at 25ºC led to a decline (~14%) in the extractable P concentration for 35 to 62 days ($P < 0.05$). A similar but less pronounced decline in Colwell P was also evident at the lower rates of SSP incorporation. A similar decline in Colwell extractable-P was evident at the highest rates of SSP application when incubated in the Kia-Ora soil, but did not occur in SSP fertilised Merrill soil.

Application of SA also increased the Colwell extractable-P concentration of the three soils, but the change in soil test value was considerably smaller than that achieved using SSP (Figs. S4 and S5). However, the Colwell P response to SA was not linear. The lowest rate of SA (2.5 g kg soil^{-1}) increased the Colwell P concentrations of all soils, but the soil test value was not increased further, or was only marginally increased by higher rates (5 and 10 g kg^{-1}) of SA application (Figs. S4 and S5).

Incubating each of the three soils with SA further increased the Colwell extractable-P concentration of the soil during the incubation period (Fig. S4; $P < 0.05$). This was in contrast to the results from incubating each soil with SSP, where there was sometimes an initial decline in the Colwell P concentration. The increase in Colwell P concentration after incubation with SA was most marked in the Merrill soil (Colwell P concentration was increased ~1.8-fold over the 172-d incubation period; Fig. S4). It is notable that the Merrill soil was also the only soil in which an initial decline in Colwell P concentration was not observed after SSP incubation.

Soil pH_{Ca} was increased marginally by adding the lowest rate of SSP to Wallaroo and Kia-Ora soils ($P < 0.05$) but was not changed further by higher applications of SSP (Fig. S6). However, the pH of the fertilised and unfertilised (control) soils declined slowly (0.2-0.3 units) over the 172-d incubation period ($P < 0.05$). Additions of SA increased soil pH linearly ($P < 0.05$) consistent with the liming value of the product, and the pH buffering capacity of each of the soils (Table 3; Fig. S4). The pH of soil to which SA had been applied also declined slowly over the 172-d incubation period, as did the unamended soils.

6.4.3. Initial fertiliser value (effectiveness) of the alternative P sources

The initial P-fertiliser value of the two products was assessed after fresh application of the products to each soil. Fertiliser value was calculated as the gradient of the yield response to P application (i.e., using data from Figure 1), but it may also be determined as the critical P requirement of the clover (i.e., the amount of fertiliser P required for 90% of maximum yield). Clear evidence of maximum shoot yields was not always able to be obtained for either product in all soils. For example, in the Wallaroo soil, the shoot yield response to SSP application was clearly asymptotic and reached a maximum yield of 1.86 g DM pot^{-1} (Fig. 1a). Shoot yield at the highest rates of SSP application to the other soils were not significantly different to this maximum yield. However, because SSP was less effective in the Kia-Ora and Merrill soils, higher rates of fertiliser application than had been used, would be needed to prove the yield asymptote had been reached (Fig. 1c, e). It was deduced that the asymptotic yield maxima in all soils should equal that achieved in the Wallaroo soil because the clover was nodulated (no nitrogen limitation) and was grown without other soil constraints (e.g., the soils were limed to eliminate potential for Al-toxicity (Table 4) and were pasteurised to avoid adverse impacts of root pathogens (Simpson et al., 2011b).

This assumption underpinned the estimates of critical P requirement in the Kia-Ora and Merrill soils and when SA was being applied.

Although shoot dry mass was increased by application of both P sources to all three soils ($P < 0.001$; Fig. 1), the effectiveness of the alternative products differed significantly. The largest initial shoot growth responses per unit P applied were achieved when the plants were grown with SSP. However, the effectiveness of SSP varied with soil type and was greatest in the Wallaroo > Kia-Ora > Merrill soils (Figs. 2a and 3a). The initial gradient of the yield response to P was highly and negatively related with the soil PBI ($R^2 > 0.90$; Fig. 2a). Conversely, critical SSP requirements were positively correlated with soil PBI ($R^2 > 0.82$; Fig. 2c).

Applications of SA were substantially less effective than SSP for shoot growth in all three soils when compared at equivalent rates of total P application (Figs. 1, 2 and 3a). The relative effectiveness of SA (as determined from gradient of the yield response to total P applied) was between 22% and 64% of that achieved using SSP, depending on soil type (Fig. 3a). The fertiliser value of SA was similar and highest in the Wallaroo and Kia-Ora soils where SA effectiveness was at least 4-fold greater than in the Merrill soil. As found for SSP, fertiliser effectiveness was negatively related with PBI (Fig. 2b) and critical SA requirements positively associated with soil PBI (Fig. 2d).

Shoot dry matter yields achieved at the highest and penultimate rates of SA application in Wallaroo soil and at the highest rate of SA application in Kia-Ora soil were inhibited relative to the highest yields observed and the predicted maximum yield for *T. subterraneum* growth in these soils (Fig. 1). Yield suppression in these treatments was most likely associated with relatively high soil pH (i.e., > pH_{Ca} ~6.5) caused by the liming effect of large rates of SA application (Table S2).

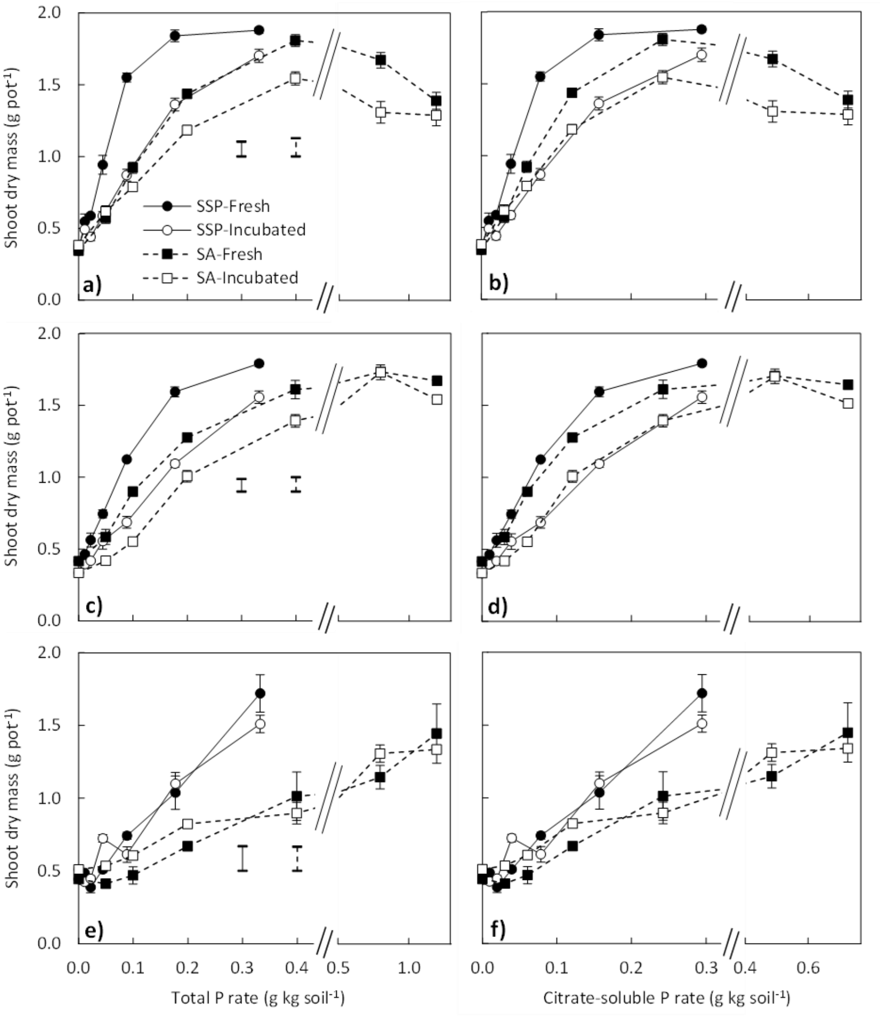

*Figure 1. Shoot dry mass of Trifolium subterraneum in response to the total amount of P (**a**, **c**, **e**) and the amount of citrate-soluble P (**b**, **d**, **f**) applied to the topsoil as single superphosphate (SSP) or sewage ash (SA) in Wallaroo (**a**, **b**; n=4), Kia-Ora (**c**, **d**; n=4) and Merrill (**e**, **f**; n=3) soils. The capped solid lines (all soils; **a**, **c** and **e**) show the LSD (P=0.05) for interaction of P application rate × incubation treatment for SSP while the capped dashed lines (**a** and **c**) show the LSD (P=0.05) for interaction of P application rate × incubation treatment for SA. In Merrill soil (**e**) the capped dashed line shows the LSD for the main effect of P application because the interaction P rate × incubation treatment for the SA application was not significant (P>0.05). Error bars associated with symbols show ± standard error.*

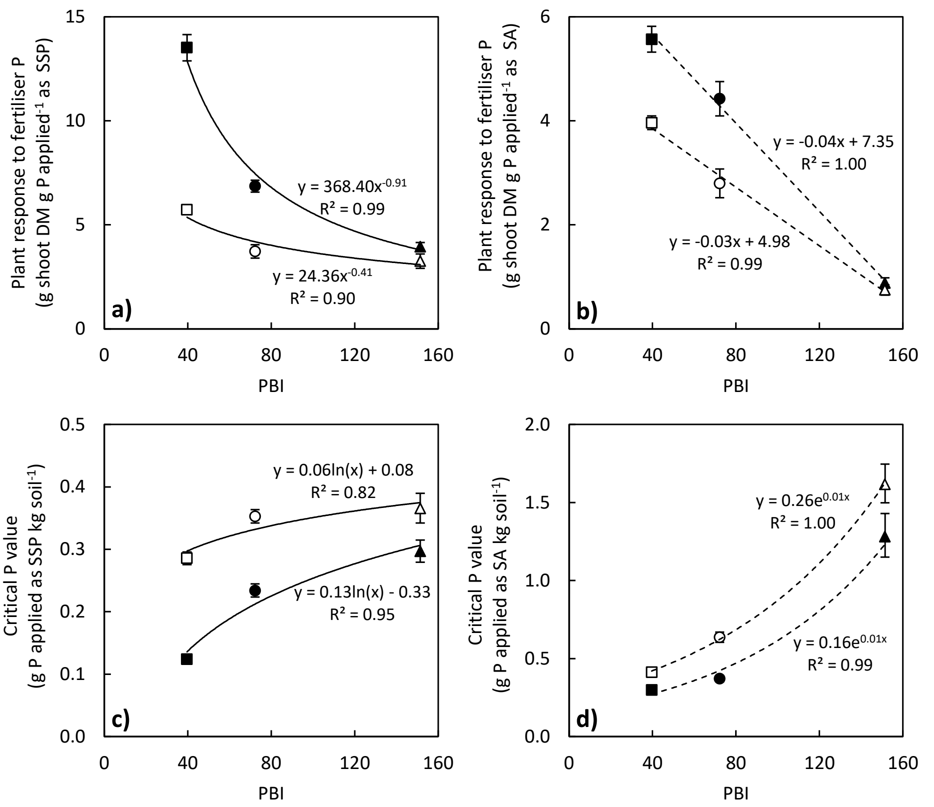

*Figure 2. Relationship between soil Phosphorus Buffering Index (PBI) and the growth response of Trifolium subterraneum expressed per unit of P applied as single superphosphate (SSP; solid lines; **a**) or sewage ash (SA; dashed lines; **b**) when applied as a fresh application (closed symbols) or after incubation (open symbols) in the Wallaroo (squares), Kia-Ora (circles) and Merrill (triangles) soils. Relationship between soil PBI and the critical P application rate (g P applied kg soil^{-1} for 90% of maximum shoot yield) for SSP (solid line; **c**) and SA (dashed line; **d**) when freshly applied (closed symbols) or after incubation with each soil (open symbols). Error bars show ± standard error.*

The soil pH and shoot yield response in the Wallaroo soil was most impacted by this phenomenon, presumably because of the lower pH buffering capacity of this soil (Table 3).

Yield responses to both fertilisers were also graphed relative to their citrate-soluble P contents (Fig. 1b, d, and f; Table 1), but this component of the fertilisers did not explain why different clover yields were achieved by each fertiliser. In contrast to SSP, SA contained no water-soluble P (Table 1), so the water-soluble P content of the fertilisers offered no explanation either.

6.4.4. Residual fertiliser value

The residual value of SSP after incubation with each soil was strongly influenced by soil type (Fig. 1). For example, after incubation in the Wallaroo and Kia-Ora soils, the residual value of SSP was only 42-54% of freshly applied SSP (Fig. 3b). In contrast, the residual value of SSP in the Merrill soil was 83% and was not significantly different (*P* <0.05) to the effectiveness of a fresh SSP application. The residual value of SA was also influenced by soil type and was substantially better (71%) than the residual value of SSP (42%) in the Wallaroo soil, and equivalent to SSP in the Kia-Ora (54-63%) and Merrill (83-85%) soils. As occurred for SSP, the high residual value of SA in the Merrill soil was not significantly different (*P* <0.05) to the effectiveness of freshly applied SA.

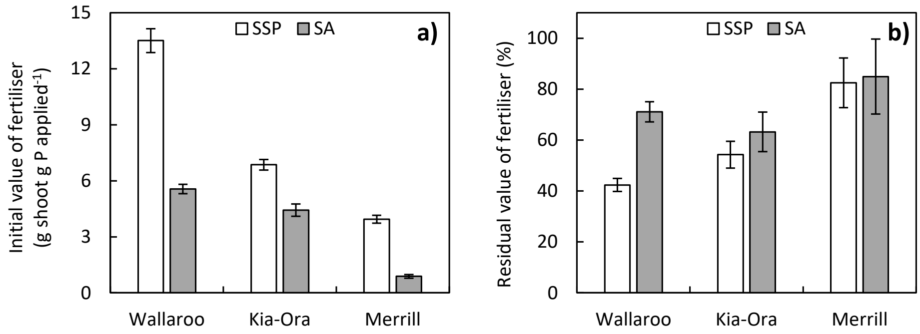

Figure 3. Initial fertiliser value (i.e., growth achieved per unit freshly applied P; ***a****) and residual value of fertiliser (i.e., growth after incubation of the fertiliser in each soil, expressed as a percentage of the growth achieved with a fresh fertiliser application;* ***b****) of single superphosphate (SSP) and sewage ash (SA). Error bars show ± standard error.*

6.4.5. Phosphorus acquisition and root acclimation in response to the P fertilisers

Phosphorus uptake and the acclimation of *T. subterraneun* roots to soil P fertility was examined in the Wallaroo soil. Total P uptake by *T. subterraneum* increased with rate of both SSP and SA (Fig 4a). P uptake was greatest in the freshly applied SSP treatment with plants continuing to acquire P after shoot yield had plateaued (i.e., "luxury" P uptake was observed). Reductions in shoot yield observed at the highest rates of SA application, or after incubation of the fertilisers with the soil, were associated with reduced P uptake. Irrespective of product type and incubation treatment, the relationship between shoot P concentration versus relative shoot yield (maximum yield = asymptote achieved with high applications of SSP) was similar. A common break-point was fitted to the broken-stick relationship (Haling et al., 2018) and indicated a critical internal P requirement of 2.8 mg P kg DM^{-1} irrespective of fertiliser treatment.

The *T. subterraneum* plants responded to shortages in P supply (as indicated by relative shoot yields <1; Fig. 5) by preferentially allocating dry matter to nutrient foraging (topsoil) roots (Fig. 5b). The ability of the plants to allocate dry matter to roots for nutrient foraging peaked at a relative yield of ~0.4. Importantly, the root acclimation response of the plants did not differ with fertiliser type or the manner (fresh versus incubated) in which a fertiliser was supplied. Subsoil roots did not respond to P supply in the same way, preferential allocations of mass to subsoil roots were only observed at the lowest rates of P supply in the topsoil (i.e., at levels of P supply where the P resources of the topsoil and subsoil would have converged) (Fig.5c). There were also no differences in subsoil root acclimation response that could be attributed to P fertiliser type.

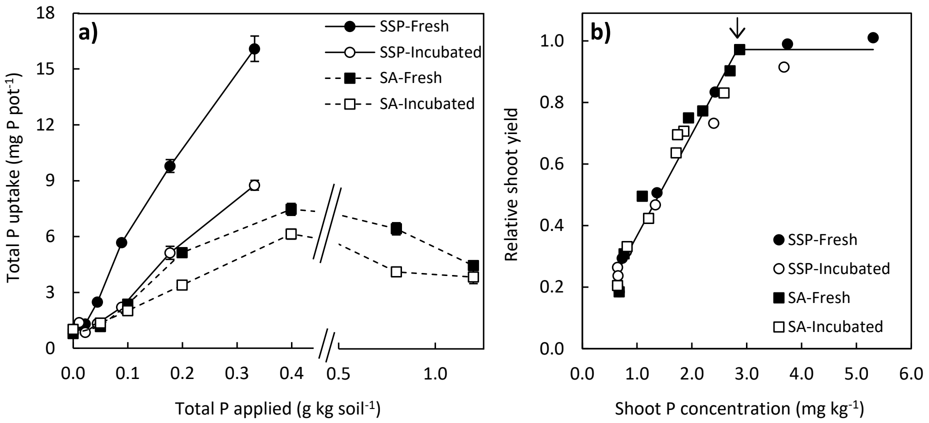

*Figure 4. Total uptake of P by Trifolium subterraneum grown in response to P applied as single superphosphate (SSP) and sewage ash (SA) either added freshly or after incubation in Wallaroo soil (**a**, n=4). Relative shoot yield graphed in relation to the shoot P concentration (mg kg dry matter^{-1}; **b**). The critical internal P concentration (i.e., P required to achieve the maximum yield) is shown by the arrow.*

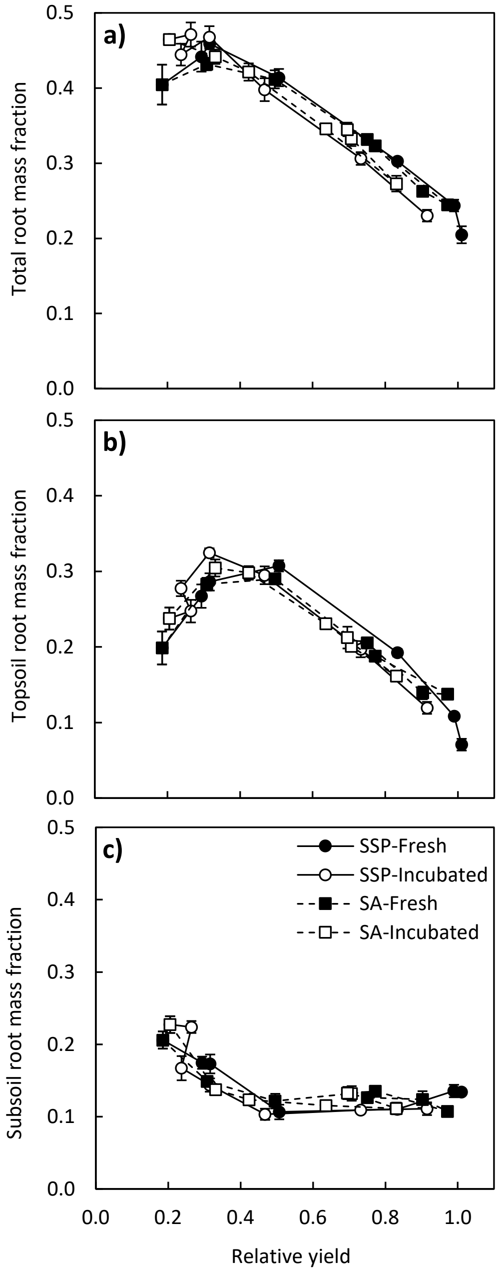

*Figure 5. Root mass fraction of the total root system (**a**), topsoil root mass fraction (**b**) and subsoil root mass fraction (**c**) of Trifolium subterraneum grown in response to single superphosphate (SSP) or sewage ash (SA) either added freshly or after incubation in Wallaroo soil, graphed in relation to relative shoot yield (n=4). Error bars show ± standard error.*

6.4.6. Soil test P response to application of the alternative P sources

The concentration of Colwell extractable P was measured in the topsoil at the beginning and at the end of the experimentation period to determine whether the availability of P for plant growth had changed markedly during the experiment. The final Colwell P (data not presented) was consistently lower, but differed by only 10-14% from the initial Colwell P. Consequently, the data are presented using the initial extractable P concentrations of the topsoil.

Clover growth in response to the Colwell P after fresh application of SSP most closely parallels the use of soil P tests for pasture management in southern Australia (Gourley et al., 2019) and was, therefore, used as the benchmark against which to compare all other soil test results. The comparison demonstrated that the Colwell P test substantially underestimated the availability of P in all soils, for clover growth after SA had been freshly applied. Plant growth responses per unit Colwell P were ~3-fold larger after fresh applications of SA, compared to the response when SSP had been applied (Figs 6a, e and i).

Incubating both fertilisers with soil also altered the plant growth response per unit Colwell P (Figs 6a, e and i). In the soils that had a measurable capacity for P sorption (i.e., the Wallaroo and Kia-Ora soils), the plant growth response per unit Colwell P declined after the fertilisers had been incubated with the soil, indicating that the extractable-P test was now tending to overestimate the availability of P. This was less obvious in the Merrill soil which had a low P-sorption capacity (as indicated by the high residual value of SSP in this soil).

The response of clover growth to the Bray-1 P test was analogous to that of the Colwell P test (Figs 6c, g and k).

The clover yield responses to $CaCl_2$-P after fresh applications of SSP were similar in all three soil types (Figs 6b, f and j) and the responses to $CaCl_2$-P after applications of SA appeared to be similar to that of freshly applied SSP at the lowest fertiliser application rates. However, at higher fertiliser application rates, clover yield per unit $CaCl_2$-P for SA applications departed from the yield per unit $CaCl_2$-P for SSP applications and underestimated the availability of P from SA.

Predictably, the Colwell P, $CaCl_2$-extractable P and Bray-1 tests detected lower plant P-availability for a given unit of total P when P was supplied as SA compared with SSP (Fig. 6 insets). Bray-1 P also underestimated the availability of P after SA application to soil, relative to that observed when SSP had been applied to soil. In contrast to the other extractable-P tests, the BSES soil P test extracted similar amounts of P for a given unit of total P applied regardless of the P treatment.

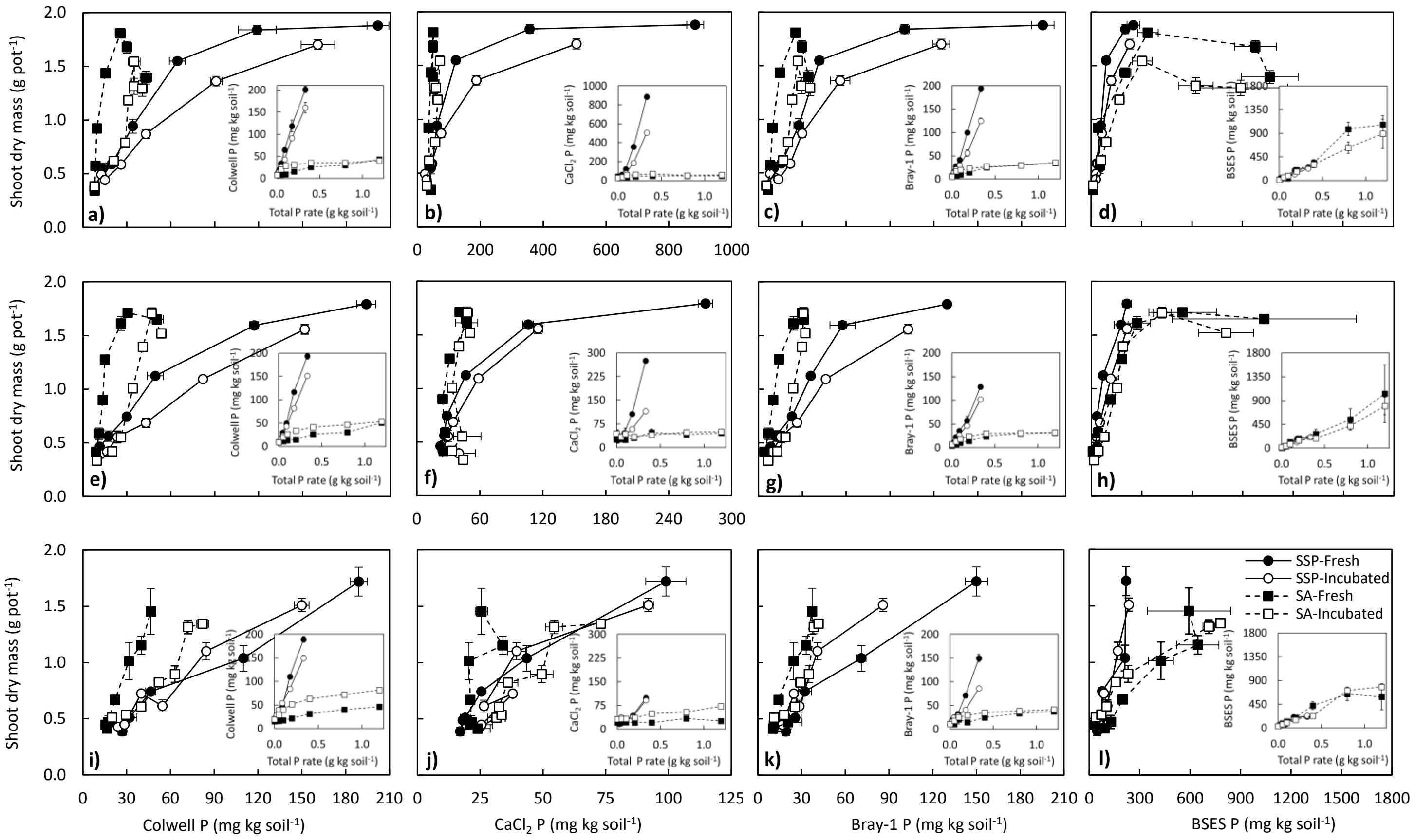
Shoot dry mass (g pot-1)
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
Colwell P (mg kg soil-1)
CaCl2 P (mg kg soil-1)
Bray-1 P (mg kg soil-1)
BSES P (mg kg soil-1)
Total P rate (g kg soil-1)
SSP-Fresh
SSP-Incubated
SA-Fresh
SA-Incubated
0 30 60 90 120 150 180 210
0 25 50 75 100 125
0 200 400 600 800 1000
0 60 120 180 240 300
0 300 600 900 1200 1500 1800
0.0 0.5 1.0 1.5 2.0

*Figure 6. Relationship between the Trifolium subterraneum shoot dry mass and Colwell P (**a**, **e**, **i**), CaCl$_2$-P (**b**, **f**, **j**; NOTE: different scale on the y-axis for these panels), Bray-1 P (**c**, **g**, **k**) and BSES P (**d**, **h**, **l**) in Wallaroo (**a**, **b**, **c**, **d**), Kia-Ora (**e**, **f**, **g**, **h**) and Merrill (**i**, **j**, **k**, **l**) soils. Insets show the relationship between the soil P test values and the rates of total P applied to the topsoil after application of single superphosphate (SSP) or sewage ash (SA). Error bars show ± standard error. Legend to all panels is shown in panel (**l**).*

6.5. Discussion

6.5.1. The fertiliser value of freshly applied product

Product P content and solubility: Farmers contemplating using SA as a P source for pasture production must contend with issues that arise less frequently when using a soluble P fertiliser, such as SSP, that has been manufactured to meet a standard composition specification. The total P content of the SA from the source used in the present experiments is known to vary between batches. In early experiments, the product contained 32 mg P kg^{-1} and was mainly considered to be valuable as a liming material (Dann et al., 1989). In more recent times, the product has contained 66 mg P kg^{-1}, of which 11.2 mg P kg^{-1} was citrate-soluble (Leech et al., 2019), and 80 mg P kg^{-1}, of which 48.5 mg P kg^{-1} was citrate-soluble (i.e., the present experiment). Importantly, the proportion of total P that was citrate-soluble in these latter samples of SA varied substantially (range: 17%-61% of total P in the product).

Leech at al. (2019) assessed the fertiliser value of a range of alternative P sources and suggested the initial fertiliser value of a product can often be predicted from the proportion of P that is citrate-soluble. In the current experiment, we anticipated a difference in the fertiliser value of the products examined because of the large differences in solubility of P in SSP and SA. The total P content of SSP was 89% soluble in citric acid solution and 79% soluble in water. By comparison, the total P content of SA was 61% citric-acid extractable, with no water-soluble P. Consequently, shoot yield responses to citrate-extractable P were examined with the specific objective of assessing whether the citrate-soluble P content of a product would be indicative of the initial effectiveness of each fertiliser. However, the freshly applied SA treatment did not support equivalent clover growth per unit citrate-soluble P, to that achieved when using SSP (Fig. 1b, d and f). Shoot yields per unit citrate-extractable P were considerably lower in the SA treatments. This indicated that more P was extracted from SA using citric acid than was available for clover growth. Nevertheless, it is acknowledged that the duration of the present experiment (5 weeks) may only indicate the availability of P for plant growth over the short-term. It remains to be tested whether citrate-extractable P would predict P availability from SA over an entire growing season in the field.

Influence of soil P buffering: The impact of soil type on fertiliser value of freshly applied products was strongly influenced by the P-buffering capacity of each soil. The plant-availability of P for growth (as judged by initial growth response of the clover to application of P, or the critical P requirement of the clover for maximum growth) was lower in soil with higher PBI (Figs 2 and 3a). It is long established that the effectiveness of freshly applied SSP is negatively related to the ability of a soil to adsorb P from solution (Barrow, 1973). In the present experiment, the PBI measure of P-buffering (a single-point representation of the curvilinear P-adsorption and buffering characteristic of a soil; Burkitt et al., 2002; 2008) was used because it is the standard soil test used to characterise soil P buffering and to predict the amount of soluble P fertiliser needed to increase soil P fertility in the field in Australia (Burkitt et al., 2001). The results from the present experiment, agreed well with the expectation that high PBI soils require greater applications of SSP to increase fertility (Burkitt et al., 2001) and to achieve maximum pasture yield (Gourley et al., 2019). Importantly, they also indicated that the plant-availability of P from freshly applied SA was influenced in an analogous manner and was lower in soils with higher PBI.

We tested whether our reliance on plant growth responses to each P source was a reasonable reflection of P availability by examining the relationship between relative shoot yield and shoot P concentration when the clover was grown with freshly applied SSP or SA. The relationships and predicted critical internal P concentrations were similar irrespective of the form in which P was supplied (Fig. 4b). Likewise, the relative allocations of mass to nutrient foraging (i.e., topsoil) roots were similar when the treatments were compared at equivalent levels of P-stress (as indicated by relative shoot yield) (Fig. 5). The allocation of plant dry mass to nutrient foraging is a universal plant acclimation response to P stress

(Freshet et al., 2015; Haling et al., 2018). The similarities in internal P concentrations and root morphology responses among the P supply treatments indicated: (i) that shoot yield was an accurate surrogate measure of shoot P uptake, and (ii) that differences in yield among the P supply treatments were most likely due to differences in P availability, rather than any other potential inhibitory effects of the products or the rate and manner of their supply. This became important, for example, when assessing why very high rates of SA where inhibitory for plant growth (Fig. 1) and P uptake (Fig. 4a) in the Wallaroo soil. The constraint to growth was associated with soil pH rising above pH 6.5 (Table S2). However, because the internal P concentrations of the clovers and their root morphology responses to P stress in these treatments were consistent with P stress caused by low P supply, we deduce that the inhibitory impact of high SA supply was due to high soil pH constraining the availability of P for growth; a phenomenon that has been noted elsewhere (Dann et al., 1989; Curtin and Syers, 2001; Barrow et al., 2020).

6.5.2. Residual fertiliser value

After the initial phosphate buffering reactions that occur when soluble phosphate is applied to a soil, phosphate continues to react slowly with the soil resulting in a declining residual fertiliser value over time (Barrow, 1974; Barrow and Shaw, 1975; Bolland and Gilkes, 1998a, b). We incubated the fertilisers with each soil at 25ºC for about 3 months to hasten the slow reactions (Barrow, 1983, 1999) and then used the incubated soils to measure residual fertiliser value (Figs 1 and 3). The residual value of SSP was 42% and 54% in the Wallaroo and Kia-Ora soils, respectively (Fig. 3b). Residual values of this magnitude are similar to those that have been recorded about one year after applications of SSP to soils used in Australian agriculture (e.g., Bolland, 1986; Bolland et al., 1987, 1988). In contrast, SSP incubated with the Merrill soil had a high residual value (~82%; Fig. 3b), that did not differ significantly from the initial fertiliser value of the freshly applied SSP. This indicated a soil with a low propensity to sorb P. The differences in initial and residual values of SSP among the three soils selected for the experiments confirmed that we had successfully selected soils with contrasting phosphate-buffering and phosphate-sorption characteristics for the fertiliser application experiment.

The residual P value of SA after its incubation with each of the three soils was between 63% and 85%, with no significant differences among the soils (Fig. 3b). The high residual value of SA in the Merrill soil was also not significantly different from the fertiliser value of freshly applied SA. Given that the Merrill soil had a low capacity for P sorption, this latter observation was predictable. However, SA also exhibited a relatively high residual value in the Wallaroo soil which had a relatively high P-sorption capacity. On face value, the contrasting residual values for SSP and SA in this soil appeared to be inconsistent. However, there are potential reasons why SA may deliver relatively good residual value, even in soils with a high P-sorption capacity:

(i) The opportunity for phosphate sorption from SA should be considerably less than that of a soluble-phosphate fertiliser (e.g., SSP) because the rate of P sorption in a soil (pH <7) is directly proportional to the concentration of phosphate in soil solution (Barrow, 1980a, b). The low solubility of P in SA (Table 2) would ensure low phosphate concentrations in soil solution.

(ii) Sewage ash is also expected to release phosphate slowly to soil solution over time. Slow release of plant-available P from SA was indicated in the present experiments by the fact that the Colwell extractable-P concentration of the three soils increased over time when SA was incubated with each soil, in contrast to the initial decline in Colwell P concentration of soils incubated with SSP (Fig. S4).

Our hypothesis that the slow P-sorption reactions would determine the residual fertiliser value of SA, as it does for soluble-P fertilisers, was not supported by the fact that the residual value of SA was not significantly different in all three soils. It remains likely that the P sorption characteristic of a soil modifies the residual value of SA. For example, it was noteworthy that increases in the Colwell extractable-P concentration of soil incubated with SA were largest in the Merrill soil which had the lowest low P sorption capacity. However, the relatively high residual value of SA among all soils may indicate that physicochemical factors governing the rate of slow P release from SA were also important.

6.5.3. Relationships between extractable-P soil tests and clover yield

Soil fertility monitoring systems depend on having soil tests capable of estimating the quantum of P available for crop uptake. Many different extraction methods (e.g., Olsen P, Colwell P, Bray-1 P, etc.) are used for this purpose. The expectation is that soil tests may not detect the actual amount of P that is plant-available, but they must extract a fraction of soil P that is correlated with the amount of P that can be acquired by a crop. This enables soil test values to be used as a guide to soil fertility management for crop production (Bolland et al., 1989; Jordan-Meille et al., 2012).

We examined four contrasting soil test methods to determine whether they were likely to predict the response of subterranean clover to applications of SA (Fig. 6). We used the Colwell extractable P soil test (Colwell, 1963) and the clover growth response to freshly applied SSP as pragmatic benchmarks for this comparison because the Colwell P test is the index of soil P availability used most commonly in Australia (Gourley et al., 2019), and SSP is the preferred P fertiliser for legume-based pastures. The initial gradients of the shoot growth responses were examined to assess the ability of each soil test to detect plant-available P. It was anticipated that the plant growth response per unit Colwell P after SSP had been freshly applied (i.e., differences in the initial gradients of the relationships shown in Figs 6a, e and i) would vary with soil PBI. Indeed, larger plant growth responses per unit Colwell P were observed when PBI was lower. This reflected the fact that pasture and crop responses to Colwell P also vary predictably with the P-buffering capacity of a soil (Helyer and Spencer, 1977; Moody, 2007; Gourley et al., 2019).

Unfortunately, no soil test provided a universal explanation for the shoot growth achieved among the fertiliser treatments. When the initial shoot yield response per unit Colwell P test after fresh-application of SSP was used as a pragmatic reference point, it was clear that the Colwell P, $CaCl_2$-P and Bray P soil tests would underestimate the availability of P for plant growth when SA was used as a fertiliser (i.e., plant growth per soil test unit was usually greater after application of SA, than when SSP had been applied). This indicated that all of the soil tests would need to be specifically re-calibrated to predict pasture growth in response to applications of SA. It was also apparent that most of the soil P tests overestimated the residual plant-availability of P from both SA and SSP. These results reflect conclusions from other studies that have also failed to find a soil test method that universally predicts the fertiliser value of SA or other ash products (Bierman and Rosen, 1994; Pagliari et al., 2010). Indeed, Brod et al. (2015) concluded that the choice of soil test method depended on the pH of the soil, and whether the aim was to predict the initial value of the fertiliser or its late-season value.

The least useful of the soil tests was BSES P. Superficially, the clover responses to BSES P concentrations appeared to be more similar among the various P application treatments, particularly in the Wallaroo and Kia-Ora soils. However, when the soil test values were graphed relative to the amounts of total P applied (see Fig. 6 insets), it was apparent that this soil test (a 0.005 M H_2SO_4 extract of the soil) had extracted a fixed proportion of the total P applied in each of the P application treatments, irrespective of the P product being used, or its manner of application.

6.6. Conclusions

There is clear evidence that SA can be used as a P fertiliser for crop and pasture growth, and it is imperative from a global resource conservation perspective that this should occur. This form of recycled effluent P has several advantages over other recycled sewage products as outlined previously. However, the use of SA also incurs a number of challenges that impact its applicability. In this paper, we focussed on how SA reacts with three soils typically used for pasture production, where SA may potentially be used as an alternative to SSP. These soils are very acid (pH_{Ca} <5), potentially aluminium toxic, P deficient, and likely to accumulate large proportions of the P that is applied as fertiliser.

In summary the key agronomic challenges in using SA, relative to the use of a soluble P fertiliser such as SSP, included:

- Low P-solubility of SA meaning that larger quantities (2- to 3-fold as much product) must be applied to achieve similar initial pasture yield improvements.
- Larger quantities of SA were required in soils with a higher P buffering. This was analogous to the impact of PBI on plant-availability of P and the higher rates at which soluble P fertiliser must be applied to increase soil test P. It indicates a need to provide farmers with guidelines that link the initial fertiliser effectiveness of SA with the PBI characteristic of a soil.
- In soils with low soil pH buffering capacity, large rates of SA application may also increase soil pH to levels that inhibit the plant-availability of P. Consequently, there is a need to understand the liming value of the SA product, the pH buffering capacity of the soil to which it is being applied, and the soil pH tolerance of the pasture species under cultivation.

In the present experiment, the degree to which P in SA was “plant-available” was not predicted by either the water-solubility, or citric acid-solubility of P in the SA product. A measure of the initial plant-availability of P in SA is needed by farmers aiming to calculate rates of fertiliser application. It was equally concerning that common soil test extractants underpredicted that availability of P from SA for clover growth relative to P availability from SSP. This implies a need to "recalibrate" soil tests for use with SA and potentially presents a major problem for soil P management if farmers choose to use products such as SA and SSP interchangeably.

A significant beneficial feature of SA was the high residual P value of the fertiliser. The present experiment indicated that the residual P value of SA was relatively high in all three soils and was not predicted well by any one of the measured soil P or soil chemistry characteristics alone. We surmise that this is because a number of factors contribute to the realised residual P value of SA in each soil, including the rate of P release from SA (soil chemistry and soil microbiology influences), the soil's P buffering characteristic, the soil's capacity for P sorption (fixation), and the influence of soil pH on P availability. The true residual value of SA should be further characterised in longer-term field experiments because there is an important "trade-off" for farm use between the low initial P-fertiliser value of SA (requiring high initial application rates) and its residual P-value. The present results suggest that the most effective use of SA may occur in soils that have low to moderate P-buffering capacity (therefore, relatively high initial fertiliser value) combined with a low-moderate P-sorption capacity to assist the retention of a high residual P value. This conclusion is partially supported by field experiments (Leech et al., 2019) where single, large applications of SA have proven, after several years, to be relatively cost-effectiveness when compared with conventional fertiliser practice where smaller annual applications of SSP are used.

Acknowledgements

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Data availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

6.7. Supplementary information

Table S1. Nutrient treatments of the subtracted-nutrient experiment. The nutrients were mixed throughout the topsoil prior to it being added to each pot.

		ALL+lime[a]	**ALL**	**-P**	**-K**	**-S**	**-Micros**	**Nil**
$CaHPO_4$	mg kg^{-1}	1318	1318	0	1318	1318	1318	0
$MgSO_4 \cdot 7H_2O$	mg kg^{-1}	119	119	119	119	0	119	0
K_2SO_4	mg kg^{-1}	198	198	198	0	0	198	0
KCl	mg kg^{-1}	0	0	0	0	168	0	0
$CaSO_4 \cdot 2H_2O$	mg kg^{-1}	0	0	0	371	0	0	0
$CaCl_2 \cdot 2H_2O$	mg kg^{-1}	115	115	115	0	0	115	0
$CaMg(CO_3)_2$	mg kg^{-1}	0	0	0	0	89	0	0
$CaCO_3$	mg kg^{-1}	50	50	50	50	0	50	0
Fe-EDTA	mg kg^{-1}	1.29	1.29	1.29	1.29	1.29	1.29	0
NH_4NO_3	mg kg^{-1}	14	14	14	14	14	14	14
Micronutrients[b]	ml kg^{-1}	0.07	0.07	0.07	0.07	0.07	0.07	0

[a] The amount of lime differed with soil type: 2031 mg kg^{-1}, 2371 mg kg^{-1} and 6500 mg kg^{-1} for the Wallaroo, Kia-Ora and Merrill soils, respectively. This was sufficient lime to achieve pH_{Ca} 5.5

[b] The micronutrient stock solution contained: 1422 mg l^{-1} H_3BO_3, 9104 mg l^{-1} $MnCl_2 \cdot 4H_2O$, 4313 mg l^{-1} $ZnSO_4 \cdot 7H_2O$, 399 mg l^{-1} $CuSO_4 \cdot 5H_2O$, 865 mg l^{-1} $(NH_4)_2MoO_4$, 238 mg l^{-1} $CoCl_2 \cdot 6H_2O$

Table S2. Soil pH_{Ca} *values of topsoil layer at start and at the end of the main pot experiment after applications of single superphosphate (SSP) or sewage ash (SA). The standard errors are reported in the brackets.*

	Wallaroo		**Kia-Ora**		**Merrill**	
SSP application rate (g kg soil^{-1})	Start (n=2)	End (n=4)	Start (n=2)	End (n=4)	Start (n=2)	End (n=3)
Fresh						
0.00	6.24 (0.04)	6.53 (0.02)	5.71 (0.03)	5.67 (0.03)	5.60 (0.04)	5.97 (0.02)
0.11	6.36 (0.02)	6.49 (0.04)	5.85 (0.02)	5.84 (0.02)	5.70 (0.07)	5.98 (0.07)
0.23	6.22 (0.04)	6.44 (0.03)	5.75 (0.02)	5.70 (0.01)	5.62 (0.09)	5.85 (0.10)
0.45	6.20 (0.02)	6.29 (0.02)	5.66 (0.01)	5.66 (0.02)	5.54 (0.04)	5.85 (0.05)
0.91	6.14 (0.03)	6.21 (0.04)	5.76 (0.02)	5.64 (0.03)	5.41 (0.06)	5.85 (0.01)
1.82	6.02 (0.02)	6.11 (0.03)	5.74 (0.00)	5.66 (0.02)	5.68 (0.08)	5.78 (0.03)
3.41	5.82 (0.01)	6.03 (0.02)	5.62 (0.04)	5.62 (0.02)	5.57 (0.07)	5.77 (0.05)
Incubated						
0.00	5.99 (0.00)	6.20 (0.07)	5.68 (0.02)	5.86 (0.02)	5.72 (0.03)	5.98 (0.02)
0.11	6.44 (0.04)	6.62 (0.08)	5.80 (0.01)	5.94 (0.03)	5.55 (0.04)	5.82 (0.05)
0.23	6.14 (0.06)	6.42 (0.06)	5.64 (0.02)	5.85 (0.01)	5.53 (0.02)	5.80 (0.03)
0.45	6.15 (0.02)	6.33 (0.04)	5.64 (0.02)	5.79 (0.03)	5.61 (0.02)	5.82 (0.03)
0.91	6.44 (0.01)	6.35 (0.06)	5.59 (0.01)	5.72 (0.05)	5.42 (0.01)	5.80 (0.05)
1.82	6.08 (0.02)	6.25 (0.02)	5.66 (0.01)	5.71 (0.04)	5.60 (0.05)	5.79 (0.02)
3.41	5.88 (0.03)	6.14 (0.02)	5.61 (0.01)	5.72 (0.01)	5.53 (0.03)	5.76 (0.04)
LSD Fertiliser rate				0.05	0.11	0.09
LSD Incubation				0.03		
LSD Fert.rate x Incubation	0.09	0.13	0.06			
SA application rate (g kg soil^{-1})						
Fresh						
0.00	6.24 (0.04)	6.53 (0.02)	5.71 (0.03)	5.67 (0.03)	5.60 (0.04)	5.97 (0.02)
0.63	6.02 (0.02)	6.24 (0.05)	5.62 (0.04)	5.57 (0.02)	5.73 (0.06)	5.90 (0.02)
1.25	5.93 (0.05)	6.06 (0.02)	5.60 (0.05)	5.62 (0.03)	5.56 (0.01)	5.86 (0.05)
2.50	5.90 (0.01)	5.97 (0.02)	5.68 (0.02)	5.66 (0.01)	5.62 (0.06)	5.73 (0.06)
5.00	5.89 (0.01)	5.89 (0.03)	5.61 (0.06)	5.67 (0.01)	5.65 (0.02)	5.68 (0.08)
10.00	6.74 (0.04)	6.60 (0.04)	6.10 (0.02)	6.08 (0.02)	5.60 (0.05)	5.70 (0.04)
15.00	7.30 (0.01)	7.06 (0.01)	6.90 (0.02)	6.58 (0.02)	5.68 (0.04)	5.73 (0.03)
Incubated						
0.00	5.99 (0.00)	6.20 (0.07)	5.68 (0.02)	5.86 (0.02)	5.72 (0.03)	5.98 (0.02)
0.63	5.91 (0.01)	6.06 (0.02)	5.57 (0.02)	5.69 (0.02)	5.64 (0.02)	5.87 (0.01)
1.25	5.83 (0.01)	5.89 (0.01)	5.66 (0.00)	5.64 (0.02)	5.55 (0.04)	5.82 (0.04)
2.50	5.87 (0.01)	5.83 (0.04)	5.68 (0.01)	5.71 (0.04)	5.48 (0.02)	5.86 (0.01)
5.00	5.81 (0.03)	5.80 (0.02)	5.53 (0.04)	5.64 (0.02)	5.54 (0.02)	5.79 (0.01)
10.00	6.70 (0.00)	6.49 (0.05)	6.02 (0.02)	6.06 (0.02)	5.68 (0.04)	5.74 (0.03)
15.00	7.39 (0.02)	7.06 (0.05)	6.74 (0.04)	6.60 (0.02)	5.63 (0.01)	5.71 (0.04)
LSD Fertiliser rate			0.07			0.08
LSD Incubation			0.04			
LSD Fert.rate x Incubation	0.08	0.10		0.07	0.11	

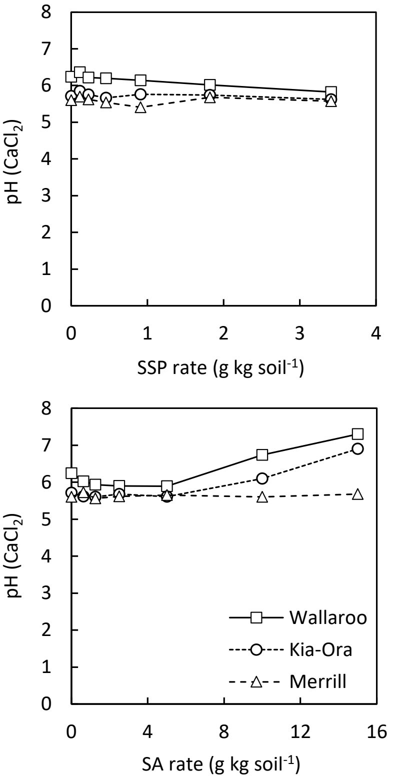

Figure S1. Soil pH$_{Ca}$ values of topsoil layer after applications of single superphosphate (SSP) or sewage ash (SA) application in Wallaroo, Kia-Ora and Merrill soils.

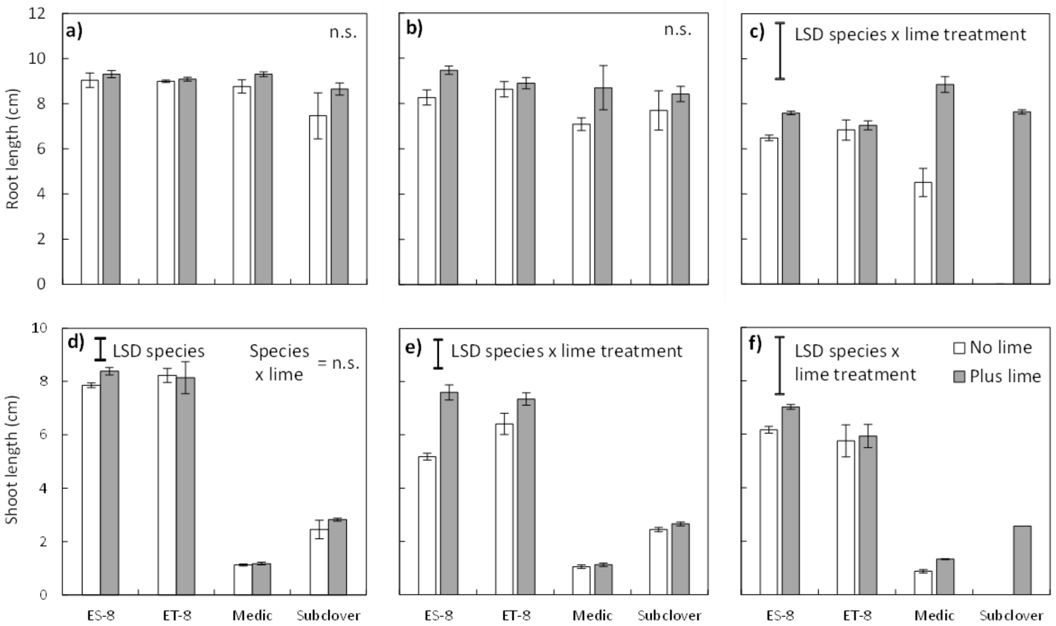

Figure S2. Root (a, b and c) and shoot (d, e and f) length of four plants: Al^{3+} sensitive line of Triticum aestivum (breeders line ES-8), Al^{3+} tolerant line of T. aestivum (ET-8), Medicago littoralis (cv. Herald) and Trifolium subterraneum (cv. Leura), when grown in each soil after lime addition to raise the pHCa to 5.5, or in the absence of lime amendment (Wallaroo soil [n=3], a and d; Kia-Ora [n=3], b and e; Merrill [n=2], c and f). The capped lines in panels c-f indicate least significant differences (LSD; P =0.05). Error bars associated with the data in all panels show ± standard error. Non significant differences are indicated by n.s. (P >0.05).

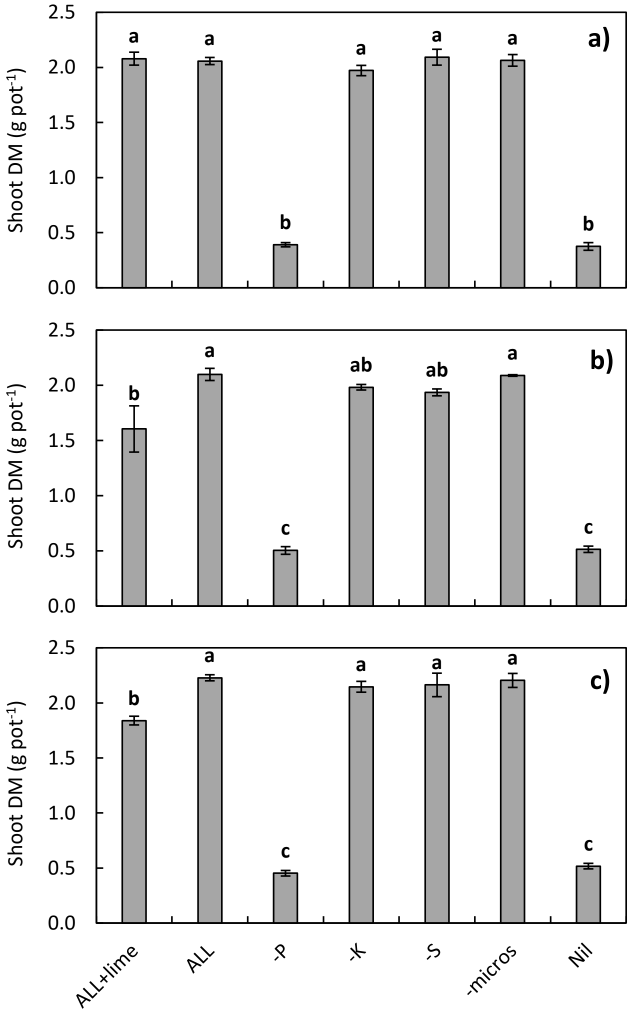

*Figure S3. Shoot dry mass per pot of Trifolium subterraneum (cv. Leura) after six weeks growth in response to different complete- and subtracted-nutrient treatments applied in the topsoil layer of the Wallaroo (**a**), Kia-Ora (**b**) and Merrill (**c**) soils. Error bars indicate ± standard error (n=4). Different letters denote significant differences among treatments within each soil type at P=0.05.*

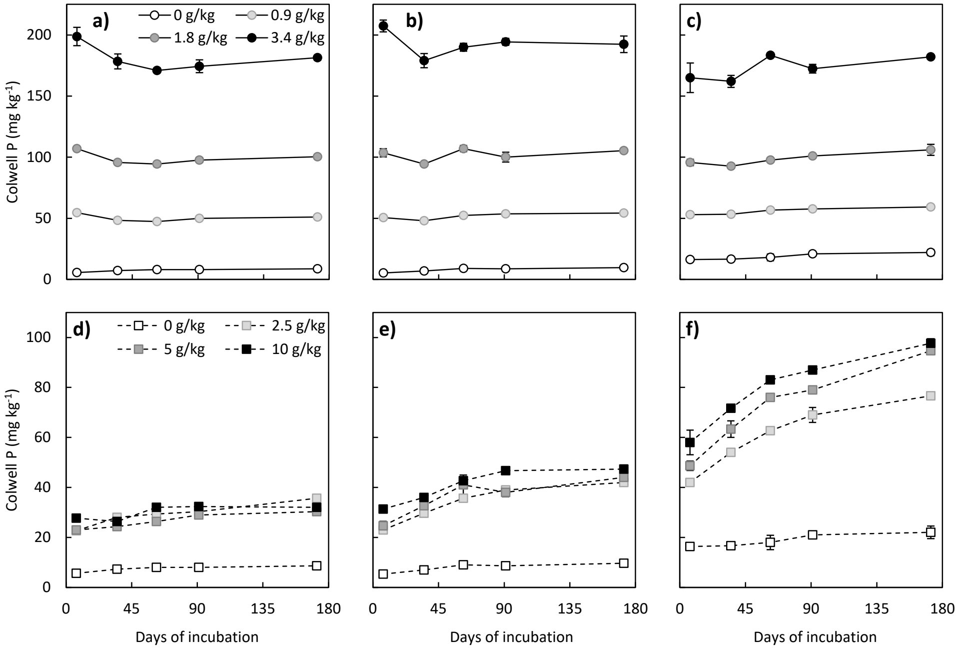

*Figure S4. Colwell P concentrations of soil at 7, 35, 62, 91 and 172 days after the application of each fertiliser product (kg ha^{-1}). Single superphosphate (**a**, **b** and **c**; circles with solid lines) and sewage ash (**d**, **e** and **f**; squares with dashed lines) were applied to the Wallaroo (**a** and **d**), Kia-Ora (**b** and **e**) and Merrill (**c** and **f**) soils. The treated soils were maintained at 25°C, with a moisture content of 70% field capacity. Error bars show ± standard error (n=3) and if not visible are smaller than the symbol.*

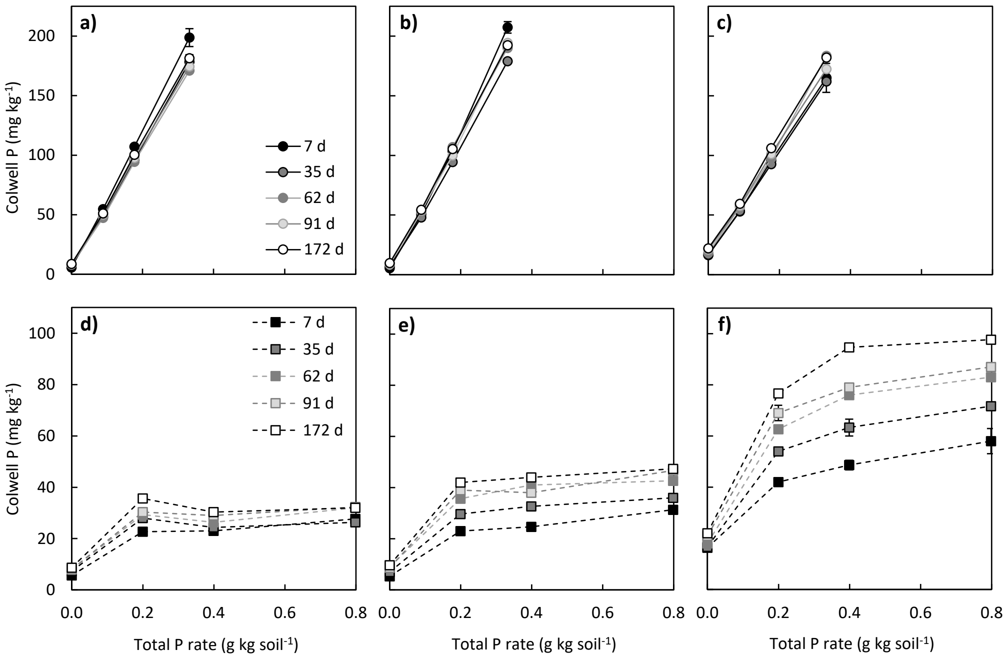

*Figure S5. Relationship between Colwell P concentrations of soil and the rate of total P application after 7, 35, 62, 91 and 172 days for single superphosphate (**a**, **b** and **c**; circles with solid lines) and sewage ash (**d**, **e** and **f**; squares with dashed lines). The products were applied to the Wallaroo (**a** and **d**), Kia-Ora (**b** and **e**) and Merrill (**c** and **f**) soils and were maintained at 25°C, with a moisture content of 70% field capacity. Error bars show ± standard error (n=3) and if not visible are smaller than the symbol.*

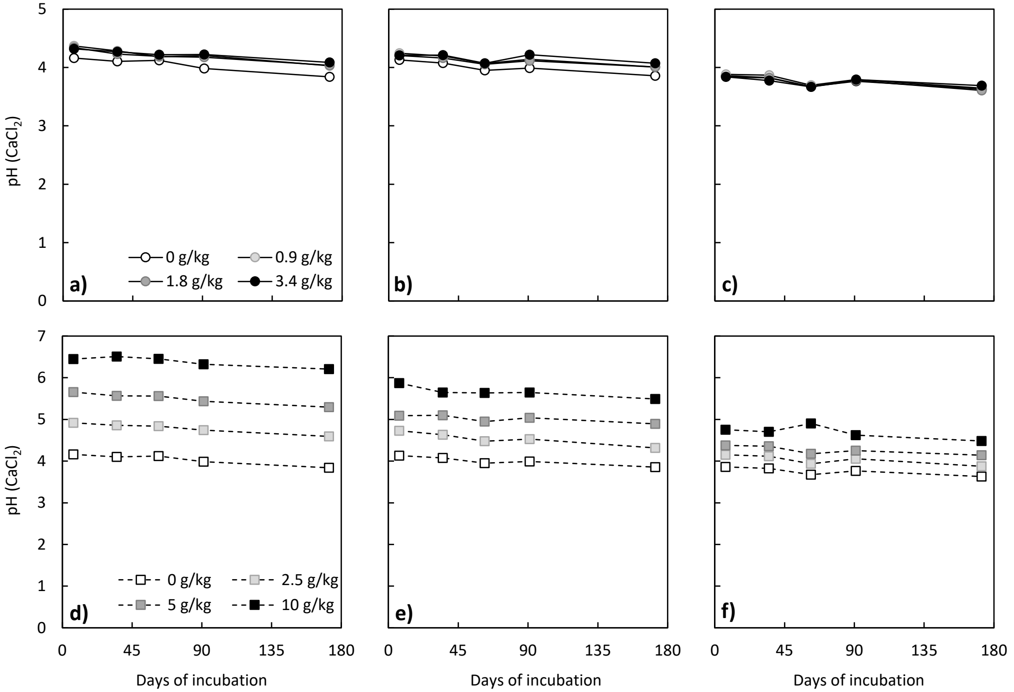

*Figure S6. Soil pH$_{Ca}$ at 7, 35, 62, 91 and 172 days after application of single superphosphate (**a**, **b** and **c**; circles with solid line) or sewage ash (**d**, **e** and **f**; squares with dashed lines) to the Wallaroo (**a** and **d**), Kia-Ora (**b** and **e**) and Merrill (**c** and **f**) soils. The treated soils were maintained at 25°C, with a moisture content of 70% field capacity. Error bars show ± standard error of mean (n=3) and if not visible are smaller than the symbol.*

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7. General conclusions

The main purpose of this PhD thesis was to analyze the potential use of improved strategies and renewable sources towards ensuring the efficient use of P fertilizers to preserve finite P resources and protect the environment.

Understanding the fate of P supplied by mineral or organic fertilizers, animal manures or other renewable sources like sewage sludge ash to farmland, and depicting the complex interactions of P with soils, other nutrients and plants, are both crucial for an economically successful and environmentally sound production of food and feed for a growing global population. In this context, a central concern of the EU agricultural policy reform was to promote the use of fertilizers in line with the circular economy model, which included the promotion of P recovered from wastes and wastewaters, and abolishing the farmers' preference of inorganic fertilizers (COM 2019/640).

When manure or P mineral fertilizer is applied to agricultural land in excess compared to the crop demand, a large amount of surplus P accumulates in soils. The legacy of past fertilizer and manure applications in soil P reserves can be used for crop nutrition during subsequent decades, even though for how long depends on how effectively the accumulated soil P is used. If repeated over many years, the soil plant-available P level can rise well above the agronomic optimum, where a further P application does not increase the yields while the risk of P losses to water bodies increases remarkably. On soils characterized by high P levels, it is desirable to use existing soil P as much as possible, thus reduce the fertilizer supply to values that do not balance the plant removal, although many years or even decades may be needed to lower the soil P levels to the agronomic optimum. The results reported in §2 confirmed that the time to observe a reduction in soil available P in the absence of fertilization can be rather long, while a build-up may occur with a limited but repeated excess of fertilization over the crop removal.

The results reported in §2 confirmed that accumulated P balance is a good and powerful predictor of soil Olsen P dynamics in the long term, making it possible to compare experiments that differ in P supply, as well as in crop, crop uptake and duration. The findings also highlighted the fundamental role that soil characteristics play in the response of soil Olsen P to different P supply sources, in particular concerning the constant level achieved in the soil as soil cumulative P balance approached $-\infty$. This observation makes clear that the P fertilizer-recommendation tools need to take different soil types into account when considering thresholds above which fertilization should be suspended. The soil Olsen P response was also influenced by P source. Specifically, P sourced from farmyard manure increased the available P pool to a greater extent than either other organics (bovine slurry, commercial organic fertilizers, green manure) or inorganic fertilizers.

Three different experiments were conducted to gather better insight to support decision makers, agronomists and farmers to sustainably intensify maize production in Northern Italy. The following questions, reported in §1.8, served as guideline to improve P use efficiency in maize cropping system:

- How can P starter fertilization be more effectively managed in the maize cropping system?
- And at the same time, can maize starter fertilization with mineral P be avoided in farm that use animal manures?
- Are there innovative strategies and sustainable alternatives to mineral P fertilization that reduce the consumption of raw materials and promote the P circular economy?

The results reported in §3 regarding maize performance after starter fertilization with P or N alone or their combination, on three different types of soil, indicate a synergistic effect of combined N and P supply on crop early growth and grain yield improvement in all the examined pedo-climatic conditions. Thus, NP starter fertilization is a crop practice that enhances the agronomic benefits of timely sowing to a great extent, and this application needs to be evaluated in broader agronomic terms, i.e. improvement of crop early development, grain yield and qualitive and sanitary grain traits rather than simply considering it as a supply to satisfy nutritional needs. In light of the findings presented in this thesis, it should be recommended to farmers who employ starter fertilization with P to consider its subsurface placement combined with N, to improve the its use efficiency. Indeed, there were limited benefits on maize crop performance from placing P-fertilizers when not combined with a N. The results showed that this practice determined benefits even in soils tested high in P (soil Olsen P ranging from 20 to 36 mg kg^{-1}). These findings were consistent with the results of §4, where the maize plant responded to NP starter fertilization with an increase in the P uptake up to the critical value of 39 mg kg^{-1} of available soil P. However, in §4, where the effects of the NP starter fertilization were evaluated in mineral or manured systems, the benefits in terms of grain yield were observed in the mineral system only, while no increase of production was recorded in systems fertilized with bovine slurry or farmyard manure. These results confirmed the use of animal manures in substitution of mineral P fertilizers is a very effective practice from the point of view of crop production.

The specialisation and industrialisation of agriculture that have geographically segregated crop and animal production systems, are important driving forces for nutrient imbalances in agriculture. To substitute the localized mineral P fertilizers at maize sowing, a new fertilization approach focuses on liquid manures or digestate injection below the seed row before planting. It is assumed that due to digestate placement the interface between nutrients and surrounding soil is markedly decreased. Thus, P absorption and/or fixation declines, resulting in a possible increased chemical and spatial availability for maize roots. Furthermore, digestate contains water-soluble P and NH_4-N, so that a similar synergic effect as described for mineral NP fertilizers is expected. The results presented in §5 show the potentials of using injected digestate as starter fertilizer for maize to reduce imports of mineral fertilizer in areas of intensive livestock farming. In analogy with the results of §4, where bovine manures were considered, digestate use might lead to an increased nutrient use efficiency compared to mineral fertiliser, without impairing maize yields and quality. The implementation of digestate or other liquid manure application technique would support several environmental goals, such as efficient use of P and N, reduction of mineral fertilizers use, preservation of limited earth P reserves, and reduced environmental pollution. Furthermore, an economic cost-benefit analysis for these alternative strategies of P fertilizations could help farmers in their decisions.

A complementary approach to resolve the increasingly difficult challenge of inadequate P supply to crops is to develop a more closed flow cycle of P resources by reducing losses from “farm to fork” and by increasing the value of recycling. In this sense, the agriculture sector makes it possible to exploit a wide range of types of wastes, also including human wastes. This favours the P cycle closure at a global scale, and allows to address the issues linked to the human waste use as a nutrient source for crops. The last study in this thesis evaluated the P fertiliser value of sewage sludge ash originated from the wastewater incineration for pasture production, on soils that differ in phosphate buffering and phosphate sorption capacity. The key questions were:

- Is the sewage sludge ash a suitable P fertilizer for *Trifolium subterraneum*?
- Is the sewage sludge ash, a less-soluble and slow-P-release source, less effective than single superphosphate, a soluble P fertilizer?

The study reported in §6 provides evidence that sewage sludge ash can be used as a P fertiliser for crop and pasture growth. However, the sewage sludge ash was a less effective fertiliser than the mineral P fertilizer, and therefore a larger amount of P (as sewage sludge ash) was needed to achieve the same yield of clover using superphosphate fertilizer. Furthermore, that study also indicates that soil tests will need to be recalibrated for use with non-conventional fertilizers such as sewage sludge ash, and point out a potential problem for interpretation of soil analyses when farmers use sewage sludge ash. This finding has broader implications for other test methods and for sewage sludge ash use elsewhere in the world.

Considerable progress has been made in understanding how certain fertilization management strategies affect the soil P availability over the long period, in which agronomic situations the adoption of the NP starter fertilization at maize sowing determines a significative improvement of crop production, and how renewable resources could be used as valuable P sources. Furthermore, the P use efficiency can be considerable increased by adopting fertilizer application techniques that enhance the plant P-acquisition. Through precise placement of mineral or organic P fertilizers close to seeds, growth and P status of crop plants can be considerably enhanced, especially during critical early growth stages.

Nevertheless, many research tasks remain to be undertaken. In particular, there are three main challenges crucial to complete the work describe in this thesis:

1. to extend the investigation, in order to identify the right placement position, relative to the seed position, of starter fertilizers - either mineral or organic - in relation to soil properties;
2. to address the lack of an analytical method really capable of assessing the soil plant available P, whatever the source and the form of P, also including the soil P legacy;
3. to develop a decision support tool to guide the P fertilization management at farm level that include all the factors that influence the soil P availability, such as the P source, the placement method, the distance from the plant roots systems and the interaction with the N and the other nutrients.

To achieve a future sustainable agriculture, it is fundamental that scientific research provides information useful for whole-comprehensive - agronomic, environmental and economic - evaluations of crop P fertilization strategies to better address farms practices.

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