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An isotope study on nitrogen and phosphorus use efficiency and movement in soil in a mimicked vermicompost-based organo-mineral fertilizer

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Abstract

Vermicompost (VC), a stabilized organic material with high organic and humic carbon, and favorable aggregation properties, was tested as a fraction of organo-mineral fertilizers (OMFs), where organic and mineral fractions interact in hotspot areas with surrounding soil. Solutions containing ^{33}P radioisotope and ^{15}N-labeled mineral fertilizers were combined with VC at two ratios of organic carbon (C_{org}) to mineral nitrogen (N) and phosphorus (P) ($OMF_{7.5C}$ and OMF_{15C}) to simulate OMF granules. Control treatments included unfertilized soil (N_0P_0), mineral fertilizer (MF_{NP}), and sole VC at two rates ($OF_{7.5C}$ and OF_{15C}). Nitrogen and P uptake by Italian ryegrass (*Lolium multiflorum*) were measured over in 8 weeks. Furthermore, MF_{NP}, $OMF_{7.5C}$, and OMF_{15C} treatments were incubated for 10 days without plant to measure atom% ^{15}N excess and ^{33}P radioactivity, as indicators of N and P movement from two soil layers (surrounding fertilizer hotspot and below it). In the pot study, OMF_{15C} caused 24% lower biomass and less nutrient recovery derived from fertilizer (N, 11% and P, 8.5%), compared to MF_{NP}. In the incubation study, OMF_{15C} exhibited +19% atom% ^{15}N excess in the combined two soil layers, relative to MF_{NP}, and +28% ^{33}P radioactivity in the soil surrounding the hotspot, and −89% in the soil below it. We interpreted this as a reduction in nutrient availability of the combined VC + mineral fertilizers, due to lower P mobility in soil. The combination of VC with mineral fertilizers can reduce P movement in soil. A higher C_{org}:N:P ratio resulted in lower nutrient use efficiency in 2 months.

Abbreviations: C_{org}, organic carbon; IC_{fert}, isotopic composition of the fertilizer; IC_{plant}, isotopic composition of the shoot biomass; N_{conc}, nitrogen concentration in shoots; Ndff, nitrogen derived from fertilizer; N_{min}, mineral nitrogen; NNI, nitrogen nutrient index; N_{opt}, nitrogen optimal concentration in shoots; N_{sol}, ^{15}N-labeled N solution; OMF, organo-mineral fertilizer; P_{conc}, phosphorus concentration in shoots; Pdff, phosphorus derived from fertilizer; P_{min}, mineral phosphorus; PNI, phosphorus nutrient index; P_{sol}, ^{33}P-labeled P solution; VC, vermicompost.

1 | INTRODUCTION

Increasing the efficiency of mineral fertilizers via enhanced recovery of fertilizer nutrients in the plants would help to reduce nutrient losses (Miao et al., 2011). Combining an organic matrix with nitrogen (N) and phosphorus (P) mineral fertilizers as organo-mineral fertilizer (OMF) can reduce N losses and increase N use efficiency (Antille et al., 2014a; Florio et al., 2016; Richards et al., 1993), increase the plant P uptake by increasing the mineral P availability, and also facilitate the application of fertilizers (Antille et al., 2013). The increase of nutrient use efficiency could be related to several processes: (i) an electrostatic attraction of mineral nutrients onto the organic material surface charges that reduce the nutrient mobility in soil (Gwenzi et al., 2018; Luo et al., 2021) that could inhibit the transformation of available nutrients into plant unavailable forms (Khiari & Parent, 2005); (ii) an increase of microbial activity that could promote an immobilization of both N and P followed by a slow nutrient release (Mandal et al., 2007), thus reducing N leaching (Richards et al., 1993); (iii) other chemical interactions between P, N, and the organic material forming new compounds with lower solubility (Carneiro et al., 2021; Luo et al., 2021; Mazeika et al., 2016); (iv) an organic material coverage of the mineral fertilizers that acts like a physical barrier to water that reduces the fertilizer solubility (Limwikran et al., 2018); and (v) an improvement of soil physical quality and consequent more suitable environment for plant production (Babalola et al., 2007).

The possible benefits of combining organic materials with mineral fertilizer in a single fertilizer will depend not only on the chemical nature of the two fractions, but also on the proportion of each fraction. According to the European legislation, OMFs can be produced with low quantities of organic C (C_{org}), just 7.5% of the total fertilizer mass (EC, 2019). As a consequence, the mass of organic materials applied to the soil will be minimal, while interaction between organic material, mineral fertilizer, and soil will be confined to hotspots in proximity of the fertilizer granules or pellets.

Currently, fossil materials like leonardite, peat, or lignite can be used as the organic fraction of an OMF (EC, 2019). These materials have the advantages of a well-established supply chain, relatively standardized among batches, stable over time, and with a high content of recalcitrant C compounds. These compounds are ascribed as the most active in improving soil properties, such as soil structure, water, and air retention (Schmilewski, 2008), and also in stimulating plant growth by the addition of humic compounds (Ayuso et al., 1996; Vujinović et al., 2020). However, peat found in peatlands, which account for 55,000 Mt C, 27% of the soil C stock in the world (Parish et al., 2008), should be protected from mining. Therefore, bio-waste materials could be explored as an alternative for the organic fraction of OMFs, reducing the destruction of these fossil C resources (Kern et al., 2017; Taparia et al., 2021).

Core Ideas

- Adding low quantities of vermicompost together with mineral fertilizers reduces phosphorus movement in soil.
- Lower P mobility in soil caused by vermicompost is linked to reduced phosphorus uptake by Italian ryegrass.
- Vermicompost has potential as an organic matrix for organo-mineral fertilizers with low organic C content.

The substitution of a raw material in an industrial process will inevitably require an adjustment in the established production process itself. The search of a new material is certainly long and difficult, also hindered by resistance to change by the industry and the market (Alexander et al., 2008), and by the need to establish a new production chain of quality-certified products. Any bio-waste material used in an OMF needs to be stable over time, homogeneous, and interact predictably with the mineral fertilizer (Bouhia et al., 2022; Sakurada et al., 2016). High and easy availability on the market, low cost, and certified technological quality are also of fundamental importance. Among possible alternatives, composting and vermicomposting biowastes can produce a stable organic material, rich in humic C, in a short time (Joshi et al., 2015; Lazcano et al., 2008; Tognetti et al., 2007).

Vermicompost (VC) alone has shown the capacity to supply nutrients to crops (Lim et al., 2015; Manivannan et al., 2009), contains bio-stimulant molecules (Xu & Geelen, 2018) that could enhance crop growth in early stages, and promotes microbial immobilization and later re-mineralization of nutrients, thus increasing availability over time (Liu et al., 2020). This makes VC a promising candidate for OMFs. Additionally, fertilizing simultaneously with VC and mineral fertilizers has shown increased nutrient use efficiency by plants (Manivannan et al., 2009; Singh et al., 2011). However, to our knowledge there are no reports of using VC as an organic matrix for an OMF with low final organic C content. It is still unclear whether low quantities of VC applied together and in proximity with mineral fertilizers could interact effectively with the mineral fertilizer and increase the fertilizer efficiency, and by which processes. Therefore, accurate measurements of the soil-crop system are necessary. Combined direct ^{15}N and ^{33}P labeling techniques in controlled conditions allow tracking accurately N and P uptake by plants from fertilizers (Bonvin et al., 2015; Traoré et al., 2020), while also studying nutrient mobility of the fertilizers in the soil that

could give insights about nutrient immobilization and losses (Frick et al., 2022; Sørensen et al., 2023).

The objective of the present study was to determine if adding low quantities of VC in close contact with mineral fertilizers—thus mimicking OMF granules—can influence mineral N and P use efficiencies and movements in soil. Italian ryegrass (*Lolium multiflorum*) was chosen as a model plant and cultivated in pots under controlled conditions. The OMF was mimicked by putting in close physical contact VC and mineral fertilizers in solution, added to the soil at amounts comparable with those of a field fertilization. Mineral fertilizers were added in concentrated solutions to ensure precise additions of labeled N and P to each experimental unit. The starting hypothesis was that VC in close contact with mineral fertilizer would result in increased nutrient efficiency of the mineral fertilizers due to a prolonged nutrient availability and reduction of nutrient losses.

2 | MATERIALS AND METHODS

2.1 | Pot experiment setup

To assess N and P uptake by Italian ryegrass, a pot experiment was carried out for 8 weeks. VC, a ^{15}N-labeled N solution (N_{sol}), and a ^{33}P-labeled P solution (P_{sol}) were used to fertilize the soil and create the different treatments. A commercial VC of bovine manure produced in Northwestern Italy was used in this study (Figure S1). The commercial VC was air-dried and milled to <2 mm. The VC was characterized using the official methods of the Regione-Piemonte (1998). The residual humidity content of the dry VC was 432 g kg^{-1}, the pH in a water suspension (1:10) was 9.9, the C_{org} value in dry matter (DM) was 198 g kg^{-1} DM, the total P was 9 g kg^{-1} DM, and the total N was 14.8 g kg^{-1} DM. Ammonium sulfate [$(NH_4)_2SO_4$] and potassium phosphate (KH_2PO_4) were used to prepare separate aqueous solution of 80.3 μg N mL^{-1} and 28.5 μg P mL^{-1}, respectively. The N_{sol} was prepared by dissolving 9.57 mg of $(NH_4)_2SO_4$ and 9.53 mg of 10 atom% $^{15}N(NH_4)_2SO_4$ into 50 mL of Milli-Q water, resulting in a N solution with 5.5 atom% ^{15}N abundance. On the same day of sowing, the P_{sol} was prepared by dissolving 625 mg of KH_2PO_4 into 50 mL of Milli-Q water, and labeled by adding carrier-free ^{33}P orthophosphate (Hartmann Analytics) solution to reach a specific activity of 10.7 kBq mg^{-1} P. Although creating a granular or pelletized OMF would have been ideal for testing potential physical interactions between VC and the mineral fertilizers, this effect was not addressed in this research because of the difficulties in producing and OMF labeled with a radioisotope P tracer. Therefore, the VC and the fertilizer solutions were used to mimicking an OMF granule by mixing them together in the soil. Treatments included two mixtures of VC with mineral fertilizers at a ratio between C_{org} –N–P_2O_5 ratio of 7.5:20:10 ($OMF_{7.5C}$) and 15:20:10 (OMF_{15C}). Controls included unfertilized soil (N_0P_0), soil fertilized with only mineral N (MF_N), only mineral P (MF_P), mineral N and P (MF_{NP}), and VC at the same rates as $OMF_{7.5C}$ ($OF_{7.5C}$) and OMF_{15C} (OF_{15C}). With the P_{min} fertilization (Figure S2), soils from the pot experiment received an activity of 314 Bq g^{-1} soil. The treatments as well as the fertilization process are described in Table 1.

The soil for the experiment was collected from the experimental station of Tetto Frati of the University of Turin in NW Italy (44° 53′ N, 7° 41′ E; elevation 245 m). Soil was collected from the first 0.2 m of the top layer of a plot managed with maize monoculture, regularly plowed and fertilized as the typical agronomic management of the area. The soil was sieved to 5 mm and air-dried for approximately 4 months prior to the start of the experiment. The soil chemical characteristics measured before the beginning of the experiment indicated a low content in both plant-available N and P (Table 2).

Before starting the pot experiment, the bulk soil was fertilized with nutrient solutions adding 300 mg K, 60 mg Ca, 50 mg Mg, 1 mg Zn, 0.1 mg Mo, 1 mg Fe, 1 mg B, 2 mg Mn, 2 mg Cu, and 0.1 mg Co kg^{-1} soil to avoid any possible complementary nutrient deficiency. After fertilization, the soil was humidified to 45% of its water holding capacity (corresponding to 109 g kg^{-1} of dry soil) and pre-incubated during 10 days at 22°C to boost soil microbial activity.

After pre-incubation, the pots were filled with the equivalent of 1 kg of air-dried soil and fertilized according to treatments. For the fertilization, two holes of 2 cm of depth and 0.5 cm of diameter were made in each pot, and on day 0, each of them was fertilized as described in Table 1. Immediately after fertilization, 0.75 g seeds of Italian ryegrass (*Lolium multiflorum* var. Gemini) were distributed uniformly over the soil and then covered with 100 g of pure sand. The pots were kept in a greenhouse at 24 and 20°C, with 12-h light, and 65% air humidity. Soils were irrigated daily based on weight loss. To satisfy the crop requirements, irrigation was increased to keep 60% of field capacity during the first 2 weeks, and then up to 70% of field capacity until the final harvest. The first harvest was made 4 weeks (Figure S3) after sowing, and a second harvest was made after 4 further weeks. The harvest consisted of cutting the whole biomass at approximately 1 cm above the soil surface.

Each treatment had four replicates. Pots were completely randomized three times per week.

2.2 | Incubation experiment setup

An incubation experiment was performed to assess the influence of the VC on the nutrient availability and flow from the mineral fertilizers in the soil. Soil fertilizers used were the same as in the pot experiment, but no plants were sown.

TABLE 1 Description of treatments; amounts of vermicompost (VC), N and P solutions (N_{sol}, P_{sol} respectively), and water applied together during fertilization; organic C (C_{org}), N and P applied through VC and mineral fertilizer in pot and incubation studies.

		Fertilization process per hole				Nutrient addition (mg kg^{-1} soil)									
						Pot experiment					Incubation				
Treatment	Description	VC (g)	Water (μL)[a]	N_{sol} (μL)	P_{sol} (μL)	C_{org}	VC N	VC P	Mineral N	Mineral P	C_{org}	VC N	VC P	Mineral N	Mineral P
N_0P_0	Control without N and P fertilization.	0	1714	0	0	0	0	0	0	0	Not applicable[b]				
MF_N	Control with only mineral N fertilization	0	914	830	0	0	0	0	133.3	0	Not applicable[b]				
MF_P	Control with only mineral P fertilization	0	1230	0	514	0	0	0	0	29.3	Not applicable[b]				
MF_{NP}	Control with mineral N and P fertilization at a ratio 20N:10P_2O_5	0	400	830	514	0	0	0	133.3	29.3	0	0	0	448.5	98.7
$OMF_{7.5C}$	Mixture of VC, N_{sol}, and P_{sol} at a ratio of 7.5C_{org}:20N:10P_2O_5	0.124	400	830	514	50.0	3.7	2.2	133.3	29.3	168.2	12.4	7.4	448.5	98.7
OMF_{15C}	Mixture of VC, N_{sol}, and P_{sol} at a ratio of 15C_{org}:20N:10P_2O_5	0.250	400	830	514	100.0	7.4	4.5	133.3	29.3	336.4	24.9	15.2	448.5	98.7
$OF_{7.5C}$	VC to provide same C_{org} as $OMF_{7.5C}$	0.124	1714	0	0	50.0	3.7	2.2	0	0	Not applicable[b]				
OF_{15C}	VC to provide same C_{org} as OMF_{15C}	0.250	1714	0	0	100.0	7.4	4.5	0	0	Not applicable[b]				

[a]First water addition was 400 μL for all treatments, the second water addition was 510 μL mimicking P_{sol}, and third water addition was 830 μL mimicking N_{sol}.

[b]Not applicable because this treatment was not included in the incubation experiment.

TABLE 2 Characterization of soil used in the pot and incubation study.

Parameter	Unit	Value
pH[a]		8.2
Total $CaCO_3$[b]	g kg^{-1}	24.0
Organic carbon[c]	g kg^{-1}	7.80
Total N[c]	g kg^{-1}	0.950
CEC[d]	meq 100 g^{-1}	8.5
Exchangeable Ca[d]	mg kg^{-1}	1292.0
Exchangeable Mg[d]	mg kg^{-1}	47.0
Exchangeable K[d]	mg kg^{-1}	85.0
Available P[e]	mg kg^{-1}	9.0
Exchangeable Fe[f]	mg kg^{-1}	27.1
Exchangeable Mn[f]	mg kg^{-1}	9.5
Exchangeable Zn[f]	mg kg^{-1}	4.1
Exchangeable Cu[f]	mg kg^{-1}	4.3

[a]In water; 1:2.5.
[b]Dietrich Calcimeter.
[c]Elemental analyzer.
[d]With $BaCl_2$ and $(OHCH_2CH_2)_3N$.
[e]Olsen.
[f]Lindsay and Norwell method.

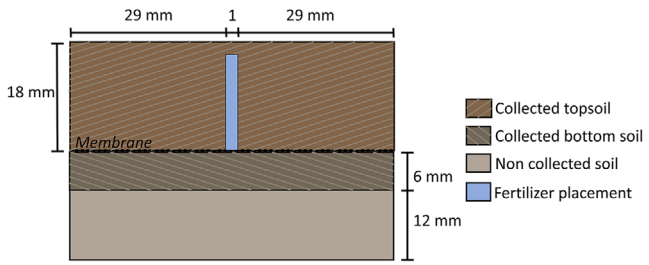

FIGURE 1 Scheme of incubation setup. Two discs were placed one above other separated by a membrane. The top disc received the fertilization. The bottom soil received nutrients through the soil solution flowing through the membrane.

The treatments for the incubation were MF_{NP}, $OMF_{7.5C}$, and OMF_{15C}.

The incubation set-up and soil sampling was adapted from Sica et al. (2023), and consisted of using plastic cylinders of 18 mm of height and 60 mm of diameter. Each experimental unit had two cylinders placed one above the another and was filled with 148.6 g of soil in total (Figure 1). The two cylinders were separated by a nylon net with 45 μm mesh size that allowed soil solution flow. The top cylinder was fertilized replicating VC, N_{sol}, and P_{sol} quantities and procedures as for one hole of the pot experiment (Table 1). On the day of the P_{min} fertilization, the P_{sol} had a specific activity of 3.5 kBq mg^{-1} P. With the P_{min} fertilization, soils from the incubation experiment received an activity of 313.5 Bq g^{-1} soil. The soil in cylinders was humidified to 70% of field capacity. Experimental units were placed in a box covered with a plastic sheet that did not allow vapor and light flows and kept at the same temperature conditions as the pot experiment for 10 days.

Each treatment had six experimental units and they were completely randomized. After the incubation, the soil from the top cylinder (topsoil) was collected entirely, while from the bottom cylinder additional soil was collected from the mesh to 6-mm depth (bottom soil). Soil from two randomly chosen experimental units was mixed to reach a higher amount of sample to be analyzed, thus leaving a total of three replicates per treatment.

2.3 | Measurements on plants

In the pot experiment, at each harvest, Italian ryegrass shoot biomass was cut and dried at 40°C for 72 h, and then weighted to calculate DM yield. Afterward, all shoot biomass was milled in a rotational miller and stored until analysis.

A chemical element analyzer (Vario Pyro cube, Elementar), coupled to a mass spectrometer (IsoPrime100 IRMS, Isoprime), was used to analyze total C, total N, and $^{15}N/^{14}N$ from shoot biomass. For determination of P concentrations in shoot tissues, 0.25 g of milled ryegrass shoot biomass were ashed at 450°C during 100 min. Subsequently, ashes were dissolved in 3 mL of 15.6 M nitric acid and then the volume was brought up to 25 mL with Milli-Q water. Total P concentration in the extracts was analyzed by colorimetry with malachite green (Ohno & Zibilske, 1991). The ^{33}P radioactivity in biomass was determined using a liquid scintillation counter (TRI CARB 2500 TR, Packard) by mixing 2 mL of extract or solution with 5 mL of a scintillation liquid (Ultima Gold AB, Packard). Values were corrected for quenching and for radioactive decay back to the day of pot fertilization.

2.4 | Measurements on soil

Soil samples of the incubation experiment were dried at 40°C for 3 days and then ball-milled and stored until analysis. Soil samples were analyzed for concentration of total N and $^{15}N/^{14}N$ ratio with the same method and instruments as for plant samples. The ^{15}N enrichment of total soil N was then related to the ^{15}N enrichment of the fertilizer and decreasing ^{15}N enrichment of soil N interpreted as less fertilizer N having moved in the respective soil zone/layer (Frick et al., 2022).

For determining P contained in soil, soil ashes were obtained similarly to plant biomass ashes. Soil ashes were dissolved in 50 mL of H_2SO_4 solution (0.5 M). Then, 5–10 mL of the solution was filtered with 0.2 μm syringe filters and stored at 4°C for 1 day until analysis of radioactivity. Values of ^{33}P radioactivity in extracts were measured 32 days after fertilization following the same procedures as with biomass

samples and corrected for radioactive decay by calculating back to day 0 of fertilization. The decrease of the specific activity of the soil P with distance from the fertilizer spot indicated decreasing presence of fertilizer P (as above explained for N).

2.5 | Calculations of nutrient indices and nutrient uptake from fertilizers

The N and P total uptake were calculated as the product between the aboveground biomass and the nutrient concentration, as shown in the following equation:

$$\text{N or P uptake (mg kg}^{-1}\text{soil)} = \text{biomass (mg DM kg}^{-1}\text{ soil)} \times \text{N or P conc (mg g}^{-1}\text{DM)} \quad (1)$$

Optimal N and P concentrations change over time, being linked to yield by a critical dilution curve. To understand if one nutrient was limiting plant growth and hence the uptake of the other nutrient, nitrogen nutrient index (NNI) and phosphorus nutrient index (PNI) were calculated, as they are more effective indicators in evaluating N and P availability than N and P concentrations alone (Duru & Ducrocq, 1996). The NNI was calculated as the ratio of the measured N concentration (N_{conc}) of the aboveground DM and the optimal N concentration in herbages, as indicated in Equation (2). The P concentration is highly linear to usual N concentrations, and therefore this relation is used to calculate the optimal P concentration for plant growth. The PNI was calculated as the P concentration (P_{conc}) ratio to the optimal P concentration, as shown in Equation (3).

$$\text{NNI} = \frac{100 \times N_{conc}}{N_{opt}} \quad (2)$$

$$\text{PNI} = \frac{100 \times P_{conc}}{P_{opt}}, \quad (3)$$

where:

- N_{conc}; P_{conc} = N and P measured concentrations (g N or P 100 g^{-1});
- N_{opt} (g N 100 g^{-1}) = Optimal N concentration, calculated as 4.8 × shoot dry matter$^{-0.32}$;
- P_{opt} (g P 100 g^{-1}) = Optimal P concentration, calculated as 0.15 + 0.065 × N_{conc}.

The direct labeling technique allowed to calculate the plant N and P recovery from the fertilizers. To do so, first the fraction of total N and P in shoots derived from fertilizer (%Ndff or %Pdff) was calculated as shown in Equation (4), using the isotopic composition of the plant and fertilizer (Barraclough, 1995; Morel et al., 1989; Traoré et al., 2020). Thereafter, the uptake of N and P derived from fertilizer (Ndff, Pdff) was calculated as shown in Equation (5), using %Ndff or %Pdff and the total N and P uptake by the plant. Finally, the N and P fertilizer recoveries (RecFertN and RecFertP) were calculated as the fraction (%) of the total N and P from fertilizer that was taken up by the shoots, as shown in Equation (6).

$$(\%\text{Ndff or }\%\text{Pdff}) = \frac{\text{ICplant}}{\text{ICfert}} \times 100, \quad (4)$$

$$(\text{Ndff or Pdff, mg kg}^{-1}\text{ soil}) = \frac{(\%\text{Ndff or }\%\text{Pdff}) \times (\text{NUplant})}{100} \quad (5)$$

$$(\text{RecFertN or RecFertP, }\%) = \frac{(\text{Ndff or Pdff})}{\text{NUfert}} \times 100, \quad (6)$$

where:

- IC_{plant} = the isotopic composition (N = atom% ^{15}N; P = kBq mg^{-1} P) of the shoot biomass at each harvest);
- IC_{fert} = the isotopic composition (Atom% ^{15}N excess; ^{33}P radioactivity = kBq mg^{-1} P) of the fertilizer;
- NU_{plant} = the total plant uptake of the nutrient (mg N or P kg^{-1} soil) at each harvest.
- NU_{fert} = the total nutrient content (mg N or P kg^{-1} soil) added through the fertilizer.

2.6 | Statistical analysis

Both experiments had a completely randomized design. When testing for differences between treatments over the harvests, a repeated measures analysis of variance (ANOVA) was used. The incubation experiment was analyzed by comparing treatments of each soil layer with a one-way ANOVA using treatment as factor. If significant differences between treatments were found, a Tukey's honestly significant difference test was performed as a post hoc comparison. Some values were analyzed as the total production (sums or averages of both harvests or both soil layers), in those cases data were analyzed by a one-way ANOVA using treatment as factor. All analyses were performed using the software R, version 4.0.5. Package multcompView was used to display post hoc results.

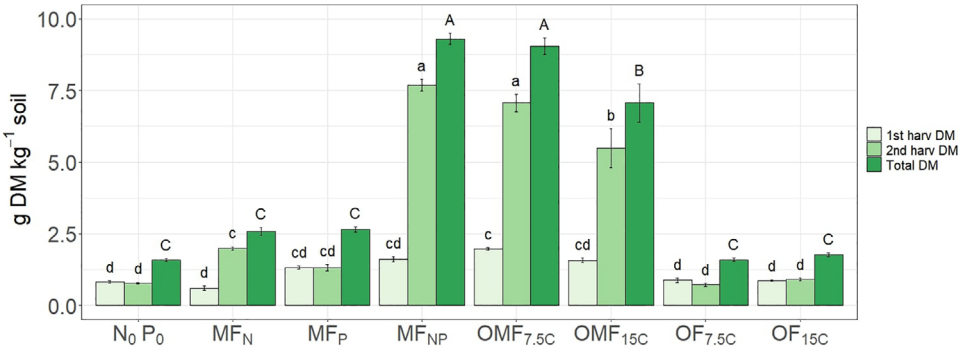

FIGURE 2 Italian ryegrass aboveground dry biomass production in the first and second harvest, and their sum. Letters above standard error bars indicate differences between treatments. Lowercase letters indicate differences between treatments and harvesting times; uppercase letters indicate differences between treatments for total biomass production.

3 | RESULTS

3.1 | Plant aboveground biomass

The two harvests of ryegrass yielded between 2 and 9 g of cumulative shoot DM (Figure 2). The addition of N_{min} or P_{min} alone did not result in an increase in cumulative DM yield compared to the unfertilized control, although a 157% increase in the second harvest yield was observed when N_{min} was added. Conversely, when N_{min} and P_{min} were combined in the MF_{NP} treatment, a strong increase in total biomass production (+434%, p-value < 0.001) was observed compared to the unfertilized control, due to an increase in the second growth period (weeks 4–8, corresponding to the second harvest).

The addition of VC alone ($OF_{7.5C}$ and OF_{15C} treatments) did not affect DM production compared to the unfertilized control, both when single harvests and cumulative yield were considered. When the mineral fertilizer and VC were combined in the OMF treatments, an increase in total biomass production was observed, compared to the control and to OF treatments (p-value < 0.001). However, the two OMF rates showed a different response compared to the mineral MF_{NP} treatment. While the $OMF_{7.5C}$ treatment responded similarly to MF_{NP}, the one at a higher organic matter rate, OMF_{15C}, produced less (p-value < 0.001). When going into the detail of single harvests, it was noted that the lower effect of fertilization with NP in combination with the high rate of VC was limited to the second harvest (−32% DM production compared to the MF_{NP} treatment, p-value < 0.001).

3.2 | Nutrient tissue concentrations, uptakes, and indices

For both N and P tissues concentrations (Table 3), differences were observed between treatments (p-value < 0.001), harvesting times (p-value < 0.001), and the interaction between these two factors (p-value < 0.001). Due to a lower plant biomass production, shoot N and P concentrations in the first harvest were consistently higher than in the second. The addition of N_{min} in the treatments MF_N, MF_{NP}, $OMF_{7.5C}$, and OMF_{15C} increased the N concentration of plant tissue in the first harvest but did not affect the plant N concentration in the second harvest, except for the P-deprived treatment MF_N. A similar effect, generally limited to the first harvesting time, was observed for P_{min} fertilization, with a longer effect of P fertilization in the N-deprived treatment. No other interaction was noticed between the two nutrients. The depressing effect on yield of the higher rate of VC in the OMF_{15C} compared to $OMF_{7.5C}$ and to MF_{NP} did not affect the N and P concentration in tissues.

The NNI and PNI (Table 3) showed that in the first harvest crops were limited by P, but not for N, while in the second harvest the limitation shifted to N, and P was taken up in excess.

The cumulative plant nutrient uptakes (Figures 3 and 4) showed a positive effect of single element fertilization on the respective element uptake, but no crossed effect (i.e., N fertilization did not increase P uptake, and vice-versa), and a strong stimulation on total nutrient uptake due to the combination of both elements. The combination of mineral fertilizers and VC was confirmed to reduce the cumulative nutrient uptakes, and in particular P uptakes of the second harvest, whereas no effect of the VC alone was observed compared to the unfertilized treatment. A high availability of N clearly enhanced the plant growth and reduced the decline of plant N uptake in the second period (weeks 4–8, as measured in the second harvesting date) compared to the unfertilized control, while P uptake was similar in the two harvests when no N was supplied, and was instead increased by the concurrent stimulation of growth caused by the addition of N.

The N and P derived from fertilizers (Ndff and Pdff, respectively) showed similar trends as the total N and P uptakes (Figures 3 and 4, respectively). Cumulative N derived from fertilizer was significantly lower in OMF_{15C} than in $OMF_{7.5C}$ and MF_{NP} (p-value < 0.001), which were similar, and this was

TABLE 3 N and P plant concentration (N_{conc} and P_{conc}, respectively) and nitrogen nutrient index (NNI) and phosphorus nutrient index (PNI) as a function of treatment and harvesting time.

Measurement	Time	N_0P_0	MF_N	MF_P	MF_{NP}	$OMF_{7.5C}$	OMF_{15C}	$OF_{7.5C}$	OF_{15C}	Average
N_{conc} (mg N g^{-1} DM)	First harvest	27.2 ± 1.5b	45.3 ± 1.9a	18.2 ± 0.7c	45.4 ± 0.5a	43.6 ± 1.3a	45.4 ± 2.1a	25.7 ± 2.5b	25.0 ± 2.3b	34.5 ± 11.0
	Second harvest	9.5 ± 0.5d	28.0 ± 2.7b	8.5 ± 0.2d	12.3 ± 2.4d	11.3 ± 1.0d	11.8 ± 1.0d	9.8 ± 0.5d	9.4 ± 0.3d	12.6 ± 6.2
	Average	18.4 ± 9.5	36.6 ± 9.5	13.4 ± 5.2	28.9 ± 17.7	27.5 ± 17.3	28.6 ± 18.0	17.7 ± 8.7	17.2 ± 8.5	
	Treatment p(F): <0.001; Time p(F): <0.001; and Interaction p(F): <0.001									
P_{conc} (mg P g^{-1} DM)	First harvest	0.9 ± 0.1 de	0.9 ± 0.0 de	1.8 ± 0.1b	2.2 ± 0.1a	2.2 ± 0.1a	2.3 ± 0.2a	1.1 ± 0.0d	1.1 ± 0.1d	1.6 ± 0.6
	Second harvest	1.0 ± 0.0 de	0.8 ± 0.0e	1.5 ± 0.1c	1.1 ± 0.0d	1.1 ± 0.1d	1.0 ± 0.1d	1.1 ± 0.1d	1.0 ± 0.1d	1.1 ± 0.2
	Average	0.9 ± 0.1	0.8 ± 0.1	1.7 ± 0.2	1.6 ± 0.6	1.6 ± 0.6	1.7 ± 0.7	1.1 ± 0.1	1.1 ± 0.1	
	Treatment p(F): <0.001; Time p(F): <0.001; and Interaction p(F): <0.001									
NNI (%)	First harvest	53.0 ± 2.2d	79.5 ± 5.2a	41.5 ± 2.6c	110.0 ± 4.0b	113.0 ± 3.9b	109.2 ± 3.6b	51.0 ± 2.1cd	49.6 ± 3.6cd	75.9 ± 29.5
	Second harvest	18.3 ± 0.9e	72.6 ± 5.9a	19.4 ± 0.6e	49.4 ± 9.8cd	44.0 ± 3.5cd	42.1 ± 3.3c	18.3 ± 0.7e	19.0 ± 0.6e	35.4 ± 19.4
	Average	35.6 ± 18.6	76.1 ± 6.3	30.5 ± 12.0	79.7 ± 33.1	78.5 ± 37.0	75.7 ± 36.0	34.7 ± 17.6	34.3 ± 16.5	
	Treatment p(F): <0.001; Time p(F): <0.001; Interaction p(F): <0.001									
PNI (%)	First harvest	44.2 ± 6.2ef	27.1 ± 0.9f	139.1 ± 11.7c	71.7 ± 2.8d	71.8 ± 2.6d	74.3 ± 3.9d	58.3 ± 4.9de	59.1 ± 3.4de	68.2 ± 31.6
	Second harvest	160.2 ± 6.6bc	42.4 ± 2.4ef	274.3 ± 20.5a	134.1 ± 20.2c	147.9 ± 3.6bc	135.3 ± 19.7c	172.0 ± 15.5b	169.5 ± 5.4b	154.5 ± 61.7
	Average	102.2 ± 62.2	34.7 ± 8.4	206.7 ± 73.9	102.9 ± 35.9	109.9 ± 40.8	104.8 ± 35.2	115.2 ± 61.7	114.3 ± 59.2	
	Treatment p(F): <0.001; Time p(F): <0.001; and Interaction p(F): <0.001									

Note: Letters indicate significant differences at $p < 0.05$ (Tukey's test) in the interaction.

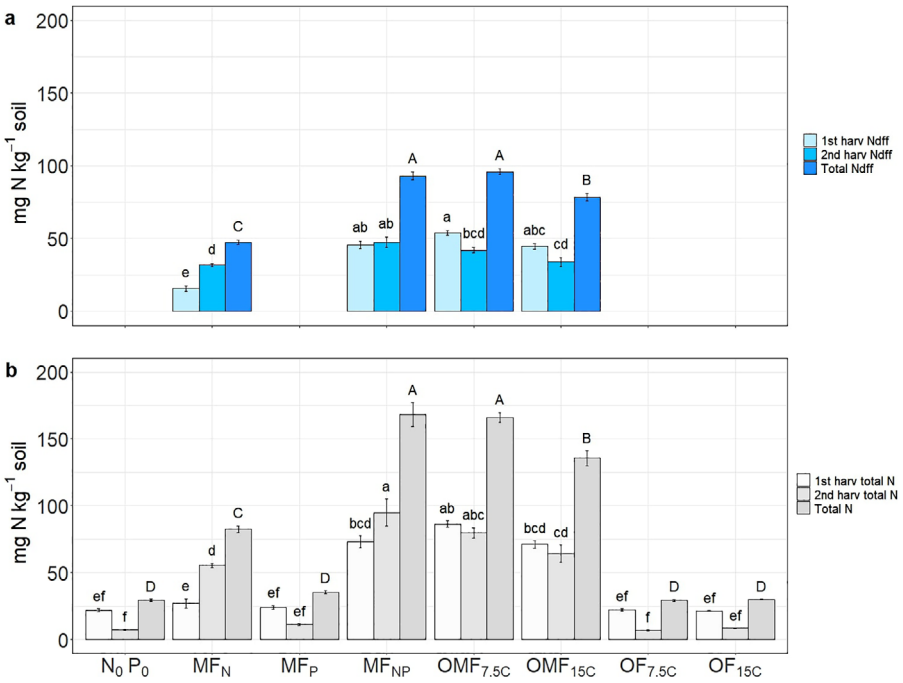

FIGURE 3 Plant N uptake in the two harvesting times, and their sum: (a) N uptake derived from fertilizer (Ndff); (b) total N uptake. Letters above standard error bars indicate differences between treatments. Lowercase letters indicate differences between treatments and harvesting times; uppercase letters indicate differences for the cumulative N and Ndff uptakes.

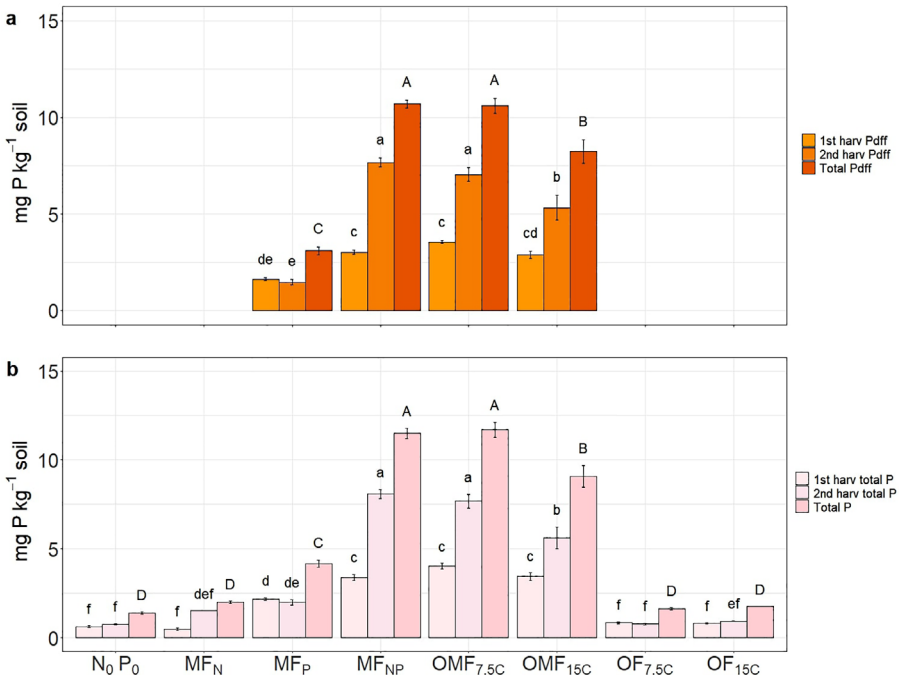

FIGURE 4 Plant P uptake in the two harvesting times, and their sum: (a) P uptake derived from fertilizer (Pdff); (b) total P uptake. Letters above standard error bars indicate differences between treatments. Lowercase letters indicate differences between treatments and harvesting times; uppercase letters indicate differences for the cumulative P and Pdff uptakes.

TABLE 4 Ryegrass labeled fertilizer N and P recovery by treatment and harvesting time.

Measurement	Time	MF_N	MF_{NP}	$OMF_{7.5C}$	OMF_{15C}	Average
Fertilizer N recovery (RecFertN)	First harvest	11.7 ± 2.9e	34.3 ± 3.9ab	40.4 ± 2.7a	33.5 ± 2.6abc	30.0 ± 11.6
	Second harvest	23.9 ± 1.7d	35.5 ± 5.3ab	31.6 ± 3.1bcd	25.4 ± 4.8cd	29.1 ± 6.0
	Treatment *p*(F): <0.001; Time *p*(F): ns; and Interaction *p*(F): <0.001					
	Total	35.6 ± 2.1C	69.8 ± 4.0A	72.0 ± 3.0A	58.9 ± 3.6B	
	Treatment *p*(F): <0.001					
Fertilizer P recovery (RecFertP)	First harvest	5.6 ± 0.5de	10.3 ± 0.7c	12.2 ± 0.6c	9.9 ± 1.2cd	9.5 ± 2.6
	Second Harvest	5.0 ± 1.0e	26.2 ± 1.6a	24.1 ± 2.4a	18.2 ± 4.4b	18.4 ± 8.8
	Treatment *p*(F): <0.001; Time *p*(F): <0.001; and Interaction *p*(F): <0.001					
	Total	10.6 ± 1.4C	36.6 ± 1.4A	36.2 ± 2.7A	28.1 ± 4.2B	
	Treatment *p*(F): <0.001					

Note: Lowercase letters indicate differences between treatments and harvesting times; uppercase letters indicate differences between treatments for the total fertilizer N or P recovery.

mainly due to differences in the second growth period (weeks 4–8). Additionally, when considering the N derived from the soil as the difference between total N uptake and Ndff, the soil provided the same amount of N for the three treatments in the first harvest. On the contrary, in the second harvest, the N uptake from soil-derived N was significantly higher in MF_{NP} than in OMF_{15C} (p-value < 0.001), with intermediate values in $OMF_{7.5C}$.

3.3 | Fertilizer recovery

The fertilizer N recovery (RecFertN) indicator followed a similar trend as Ndff uptake (Table 4). No differences in fertilizer N recovery were observed between MF_{NP}, $OMF_{7.5C}$, and OMF_{15C} (34%–40% of total N_{min}) in the first harvest, whereas in the second harvest the fertilizer N recovery in OMF_{15C} (25% of total N_{min}) was significantly lower than that of MF_{NP} (36% of total N_{min}), while $OMF_{7.5C}$ had intermediate values (32% of total N_{min}). The proportion of RecFertN increased from 12% to 24% of applied N_{min} between the first and second harvest in the MF_N treatment, remained stable around 36% in MF_{NP}, and tended to decrease in both OMF treatments (−9% and −8% for $OMF_{7.5C}$ and $OMF_{15C,}$ respectively). The overall fertilizer N recovery calculated over the 8 weeks period was 59% of the total applied N_{min}.

In the first harvest, there were no differences in fertilizer P recovery (RecFertP) between MF_{NP}, $OMF_{7.5C}$, and OMF_{15C} (10%−12% of total P_{min}), while in the second harvest, the fertilizer P recovery in MF_{NP} and MF_{15C} was significantly higher (24%–26% of total P_{min}) than in OMF_{15C} (18% of total P_{min}). The fertilizer P recovery was similar in both harvest for MF_P (5% of total P_{min}), while for treatments with combined N and P, the P fertilizer recovery increased in the second harvest (+16%, +12%, and +8% of total P_{min} for MF_{NP}, $OMF_{7.5C}$, and OMF_{15C}, respectively).

3.4 | Isotope distribution in the incubation experiment

Fertilizers with N_{min} had an atom% ^{15}N excess of 5.2%. In the incubation study, after a 10-day period, no significant differences in atom% ^{15}N excess were observed among different soil layers (Table 5). However, OMF_{15C} (2.5 atom% ^{15}N excess) exhibited a 19% higher excess in the overall analyzed soil, relative to the mineral control treatment MF_{NP}, representing the 48% of the total added N_{min}.

The ^{33}P radioactivity was higher (68%–97%) in the soil directly in contact with the fertilizer hotspot compared to the soil below it. Additionally, in comparison to MF_{NP}, the ^{33}P radioactivity in OMF_{15C} was a third higher in the soil surrounding the fertilizer hotspot. However, OMF_{15C} had almost only half of ^{33}P radioactivity than MF_{NP} in the bottom soil below the fertilizer hotspot. The total ^{33}P-specific radioactivity found in both layers represented approximately 5% of the total specific activity added through the P_{sol}.

4 | DISCUSSION

The two harvesting times, 4 and 8 weeks after fertilization, allowed us to separate short- and medium-period effects of treatments, that are probably driven by different chemical and biological processes (Bonvin et al., 2015; Oberson et al., 2010).

The soil used for this experiment had low available P and total N (Table 2), thus, resulting in a co-limitation of N and P for grass growth. This was observed in the total DM production, where treatments with only one of the mineral nutrients (N_{min} and P_{min}) did not succeed in increasing the total yield compared to the control, while adding both N_{min} and P_{min} (MF_{NP}, $OMF_{7.5}$, and OMF_{15C}) increased yield 3.5–5 times compared to the control. The need of combined N and P

TABLE 5 Isotopic distribution of N and P between two soil layers.

Measurement	Soil section	MF_{NP}	$OMF_{7.5C}$	OMF_{15C}	Average
Atom% ^{15}N excess	Topsoil	1.1 ± 0.1b	1.2 ± 0.2ab	1.4 ± 0.1a	1.2 ± 0.2
	Treatment p(F): <0.05				
	Bottom soil	1.0 ± 0.0b	0.9 ± 0.1b	1.1 ± 0.0a	1.0 ± 0.1
	Treatment p(F): <ns				
	Average[a]	1.1 ± 0.1	1.1 ± 0.2	1.3 ± 0.1	
	Treatment p(F): <0.05				
^{33}P-specific radioactivity (Bq g^{-1} soil)	Topsoil	14.2 ± 1.5b	16.3 ± 1.0ab	17.7 ± 1.6a	16.1 ± 1.9
	Treatment p(F): <0.05				
	Bottom soil	4.6 ± 2.5a	3.5 ± 1.0a	0.5 ± 0.0b	2.9 ± 2.3
	Treatment p(F): <0.05				
	Average[a]	11.8 ± 1.8	13.1 ± 1.0	13.4 ± 1.2	
	Treatment p(F): ns				

Note: Atom% ^{15}N excess: difference between atom% ^{15}N abundance—atom% ^{15}N from crop without ^{15}N fertilizers. Letters indicate differences between treatments in the same soil layer.

[a]Weighted average considering different soil mass in bottom and topsoil.

supply for this soil was also highlighted by Battisti et al. (2022) in a field study on maize.

Given the plant-growth-limiting nutrient status of the soil, the addition of an organic composted material alone did not change the situation. Although VC has been reported to provide macro- and micro-nutrients (Lim et al., 2015), in our experiment fertilization with VC alone ($OF_{7.5C}$ and OF_{15C}) did not increase biomass production (Figure 2), nor modify the N and P concentrations in shoots (Table 3) compared to the control N_0P_0. These results suggest that such low amounts of VC cannot sustain or stimulate plant biomass production. The absence of a direct fertilization effect was expected, since the amount of VC added to each treatment, equivalent to only 3.7–7.4 mg N kg^{-1} soil and 2.2–4.5 mg P kg^{-1} soil that (considering a depth of 15 cm and a bulk density of 1.3 t m^{-3}) correspond to 7–14 kg of N and 4–9 kg of P ha^{-1}, is far below the fertilization recommendation for those soils (Regione-Piemonte, 2022). As a further comparison, our study used an equivalent of 569–1138 kg VC ha^{-1} soil, while reports of using VC alone as an effective fertilizer describe fertilization rates between 5 and 20 Mg ha^{-1} soil (Joshi et al., 2015). If the absence of a direct fertilization effect was expected, a stimulating effect of VC could instead be plausible. Van Oosten et al. (2017) proposed that the plant growth biostimulant effect of humic acid-based substances in VC could be related to the differential regulation of proton ATPases located in the vacuolar and plasma membranes. Best results of using low quantities of VC as plant biostimulant were observed using VC humic extracts (Arancon et al., 2003, 2006; Atiyeh et al., 2002; Zandonadi et al., 2016), or by coating seeds with VC (Afzal et al., 2020; Amirkhani et al., 2019; Qiu et al., 2020). Humic C enhance plant growth at ratios between 50 and 500 mg humic acids kg^{-1} soil (Arancon et al., 2006; Atiyeh et al., 2002); however, in this study VC in OF_{15C} and OMF_{15C} provided only a maximum of 24.4 mg kg^{-1} soil of humic C. This observation keeps open the question of whether a higher ratio of VC to mineral fertilizer could have a biostimulant effect or not.

4.1 | Mineral fertilizers versus VC plus mineral fertilizer

The addition of mineral N and P increased the availability of these nutrients in soil and promoted plant growth. The fertilizer N and P recovery values indicate that a relatively large fraction of the fertilizer supplied was taken up by the plant. Recoveries were around 67% for N and 34% for P. A relatively low mineral P recovery was expected, as previous reports reported low fertilization P uptake given the calcareous nature of the soil used in this study (Battisti et al., 2022). Despite the plant utilized supplied nutrients and increased their concentration in tissues to a large extent (Table 3), concentrations remained rather low, if compared to optimal N concentrations in ryegrass. These values indicate that N concentrations were lower than the optimal ones in all N-unfertilized treatments and in the second cut of all treatments. MF_N was the only treatment where the deficiency of N was similar in the two growing periods. Treatments that received both N and P (MF_{NP}, $OMF_{7.5}$, and OMF_{15C}) showed some luxury consumption of N in the first cut (NNI > 100%), thus showing a limitation by P. However, in the second harvest, values were opposite and indicate that P was taken up in excess, while N was the limiting nutrient. More precisely, N was limiting in

all treatments except MF_N in the second cut, thus evidencing that the low Pdff recovery (Table 4) observed was not due to reduced P uptake, but rather to insufficient N.

Despite a similar tissue concentration, MF_{NP} and $OMF_{7.5C}$ produced more biomass in the second harvest than OMF_{15C}, thus suggesting that in both treatments, between weeks 4 and 8, there was a higher nutrient availability than in OMF_{15C}. Our results of a lower yield in OMF_{15C} are opposite to reports of field studies from Manivannan et al. (2009) and Singh et al. (2011), who found that combined fertilization of VC with mineral fertilizers increased the yield of beans (*Phaseolus vulgaris*) in clay loam and sandy loam soils; however, both reports used VC quantities several times (1250–3750 kg VC ha^{-1}) higher than our study, leading to an improvement in soil properties.

The reduction in biomass production affected the nutrient uptake. OMF_{15C} reduced the N and P uptake compared to MF_{NP} (Figure 3 and 4). A similar observation of a lower N uptake was reported in both greenhouse and field experiments with a sandy loam soil with maize using pelletized peat OMFs with a similar C_{org} content to the OMF_{15C} treatment, compared to mineral controls (Richards et al., 1993), but not in a greenhouse study with perennial ryegrass (Florio et al., 2016) growth during 4 months in 5 kg soil pots. A lower N uptake in OMF_{15C} in our experiment was also evidenced by a reduction of both Ndff and soil-derived N. This reduction of available N is not surprising, since it is known that adding organic material with a high C/N ratio reduces the concentration of available N in soil, due to microbial immobilization (Said-Pullicino et al., 2014). The VC adds available organic C to the soil and this boosts the microbial biomass, and microbes immobilize N (Guan et al., 2022). Surprisingly, these processes occurred even with the low amounts of C added in this study.

The use of OMFs could have several advantages over conventional organic or mineral fertilizers by reducing P sorption to mineral fractions (Pare et al., 2009), thus reducing the transformation of available P into plant-unavailable forms (Khiari & Parent, 2005; Parent et al., 2003), or promoting a biostimulant effect (Lee & Bartlett, 1976) that could increase early P uptake. The longer P availability should increase the P uptake by crops; however, in our results, as with N, differences in total P and Pdff uptake (Figure 4) suggest that organic materials in close contact with mineral P tend to immobilize it. The P immobilization in soil, either chemical (the soil here used is alkaline) or microbial, was enhanced by the addition of C_{org}, as also stated by Spohn and Kuzyakov (2013). The local P immobilization could explain the lower Pdff and total P uptake in OMF_{15C} compared to $OMF_{7.5C}$ and MF_{NP}. Considering the low mass of VC added to the soil, the immobilization observed in OMF_{15C} could have been caused by a localized effect of the mimicked combined fertilizer (fertilizer hotspot). Organic materials increase the microbial activity at the fertilizer–soil interface that could immobilize available N (Moritsuka et al., 2004) and P (Sica et al., 2023). The hypothesis of VC reducing nutrient availability, with a rate effect ($15C_{org} > 7.5C_{org}$), could be inferred from the results of the incubation study.

4.2 | Fertilizer recovery

The nutrient recoveries based on isotopic labeling show the fraction of the labeled N or P added to the soil that was recovered in shoots. Comparing MF_{NP}, $OMF_{7.5C}$, and OMF_{15C}, there were no differences between treatments in the first harvest for neither N nor P recovery. In the second harvest, the N and P recoveries were increased if compared to the first harvest. The N and P recoveries report a lower efficiency for OMF_{15C} than MF_{NP} in the second harvest. The reduction of recovery in OMF_{15C} is opposite to what was expected from an OMF (Deeks et al., 2013; Florio et al., 2016), where an extended availability of nutrients in time should increase the overall efficiency of fertilizers. However, the lower nutrient recovery is not necessarily an indication of lower quality for the fertilizer, as a nutrient release after 2 months could have a positive effective in long-cycle crops or after repeated fertilizer applications (Antille et al., 2014b).

Additionally, the presence of VC in OMF_{15C} can be considered to improve the fertilizer quality by reducing mineral N dispersion in the soil. This was also indicated by the higher atom% ^{15}N excess in the overall analyzed soil in this treatment, relative to MF_{NP}, that suggests a higher N retention close to the fertilizer hotspot. Florio et al. (2016), using an OMF with similar C_{org} content to the OMF_{15C} treatment, in a pot experiment found lower N leaching between 12 and 84 days after fertilization, compared to the mineral control. These results agree with the observations of the incubation experiment, where OMF_{15C} had a higher atom% ^{15}N excess in the top soil layer than MF_{NP}, thus showing that more N_{min} had remained around the VC, this potentially reducing N losses both by volatilization and leaching.

Similarly, OMF_{15C} also increased the immobilization of P in soil compared to the mineral control MF_{NP}. OMF_{15C} had in fact much lower ^{33}P radioactivity than MF_{NP} in the bottom soil below the fertilizer hotspot, which suggest that the addition of VC reduced the movement of P-labeled fertilizer. During the incubation experiment, the total radioactivity (top + bottom soil) did not change between treatments. However, OMF_{15C} had a significantly higher radioactivity in the topsoil than MF_{NP}, but it was significantly lower in the bottom soil. Those values show that adding the organic material to the mineral fertilizers reduced the mobility of Pdff in soil, which could be linked to a higher retention of the labeled solutions or to chemical interactions between P and VC. With more N

and P in the fertilizer hotspot, nutrients could remain available for crops for a longer period.

5 | CONCLUSIONS

Labeling of N and P enabled an insight of how VC in contact with mineral fertilizers retained nutrients from the mineral fertilizers, providing valuable comprehension of the fertilizer-soil-plant interactions in an idealized agricultural system. The immobilization caused by VC reduced N and P movements in soil. Such effect was higher for P than for N, resulting in a late availability of nutrients. The amount of VC was crucial to modulate the nutrient availability, because when more organic material was provided, less nutrient was plant available in the medium term. It remains unclear how the immobilization was produced by chemical or microbiological pathways. The nutrient immobilization reduced nutrient uptake by crops in a medium period of time and reduced N and P use efficiencies. Therefore, longer trials are required to understand to what extent, and in which time span VC-promoted immobilization will be reversed with a re-mineralization/release of nutrients, thus promoting and a later increase in the nutrient use efficiency by plants.

AUTHOR CONTRIBUTIONS

Tomas J. Sitzmann: Formal analysis; investigation; methodology; writing—original draft. **Pietro Sica**: Investigation; methodology; writing—review and editing. **Laura Zavattaro**: Supervision; writing—review and editing. **Barbara Moretti**: Data curation; writing—review and editing. **Carlo Grignani**: Funding acquisition; supervision; writing—review and editing. **Astrid Oberson**: Conceptualization; data curation; formal analysis; methodology; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Afzal, I., Javed, T., Amirkhani, M., & Taylor, A. G. (2020). Modern seed technology: Seed coating delivery systems for enhancing seed and crop performance. *Agriculture*, *10*(11), 526. https://doi.org/10.3390/AGRICULTURE10110526

Alexander, P., Bragg, N., Meade, R., Padelopoulos, G., & Watts, O. (2008). Peat in horticulture and conservation: The UK response to a changing world. *Mires & Peat*, *3*, 1–11. http://www.mires-and-peat.net/

Amirkhani, M., Mayton, H. S., Netravali, A. N., & Taylor, A. G. (2019). A seed coating delivery system for bio-based biostimulants to enhance plant growth. *Sustainability*, *11*(19), 5304. https://doi.org/10.3390/SU11195304

Antille, D. L., Sakrabani, R., & Godwin, R. J. (2014a). Nitrogen release characteristics from biosolids-derived organomineral fertilizers. *Communications in Soil Science and Plant Analysis*, *45*(12), 1687–1698. https://doi.org/10.1080/00103624.2014.907915

Antille, D. L., Sakrabani, R., & Godwin, R. J. (2014b). Effects of biosolids-derived organomineral fertilizers, urea, and biosolids granules on crop and soil established with ryegrass (*Lolium perenne* L.). *Communications in Soil Science and Plant Analysis*, *45*(12), 1605–1621. https://doi.org/10.1080/00103624.2013.875205

Antille, D. L., Sakrabani, R., Tyrrel, S. F., Le, M. S., & Godwin, R. J. (2013). Development of organomineral fertilisers derived from nutrient-enriched biosolids granules: Product specification. *American Society of Agricultural and Biological Engineers Annual International Meeting 2013, ASABE*, *5*, 4152–4170. https://doi.org/10.13031/aim.20131620153

Arancon, N. Q., Edwards, C. A., Lee, S., & Byrne, R. (2006). Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology*, *42*, S65–S69. https://doi.org/10.1016/j.ejsobi.2006.06.004

Arancon, N. Q., Lee, S., Edwards, C. A., & Atiyeh, R. (2003). Effects of humic acids derived from cattle, food and paper-waste vermicomposts on growth of greenhouse plants: The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002. *Pedobiologia*, *47*(5–6), 741–744. https://doi.org/10.1078/0031-4056-00253

Atiyeh, R. M., Lee, S., Edwards, C. A., Arancon, N. Q., & Metzger, J. D. (2002). The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresource Technology*, *84*(1), 7–14. https://doi.org/10.1016/S0960-8524(02)00017-2

Ayuso, M., Hernandez, T., Garcia, C., & Pascual, J. A. (1996). A comparative study of the effect on barley growth of humic substances extracted from municipal wastes and from traditional organic materials. *Journal of the Science of Food and Agriculture*, *72*, 493–500. https://doi.org/10.1002/(SICI)1097-0010(199612)72:4

Babalola, O., Oshunsanya, S. O., & Are, K. (2007). Effects of vetiver grass (*Vetiveria nigritana*) strips, vetiver grass mulch and an organomineral fertilizer on soil, water and nutrient losses and maize (*Zea mays*, L) yields. *Soil and Tillage Research*, *96*(1–2), 6–18. https://doi.org/10.1016/j.still.2007.02.008

Barraclough, D. (1995). 15N isotope dilution techniques to study soil nitrogen transformations and plant uptake. *Fertilizer Research*, *42*(1–3), 185–192. https://doi.org/10.1007/BF00750513

Battisti, M., Moretti, B., Blandino, M., Grignani, C., & Zavattaro, L. (2022). Maize response to nitrogen and phosphorus starter fertilisation in mineral-fertilised or manured systems. *The Crop Journal*, *11*, 922–932. https://doi.org/10.1016/J.CJ.2022.09.010

Bonvin, C., Etter, B., Udert, K. M., Frossard, E., Nanzer, S., Tamburini, F., & Oberson, A. (2015). Plant uptake of phosphorus and nitrogen recycled from synthetic source-separated urine. *Ambio*, *44*(2), 217–227. https://doi.org/10.1007/s13280-014-0616-6

Bouhia, Y., Hafidi, M., Ouhdouch, Y., Boukhari, M. E. M. E., Mphatso, C., Zeroual, Y., & Lyamlouli, K. (2022). Conversion of waste into organo-mineral fertilizers: Current technological trends and prospects. *Reviews in Environmental Science and Biotechnology*, *21*(2), 425–446. https://doi.org/10.1007/s11157-022-09619-y

Carneiro, J. S., da, S., Ribeiro, I. C. A., Nardis, B. O., Barbosa, C. F., Lustosa Filho, J. F., & Melo, L. C. A. (2021). Long-term effect of biochar-based fertilizers application in tropical soil: Agronomic efficiency and phosphorus availability. *Science of The Total Environment*, *760*, 143955. https://doi.org/10.1016/J.SCITOTENV.2020.143955

Deeks, L. K., Chaney, K., Murray, C., Sakrabani, R., Gedara, S., Le, M. S., Tyrrel, S., Pawlett, M., Read, R., & Smith, G. H. (2013). A new sludge-derived organo-mineral fertilizer gives similar crop yields as conventional fertilizers. *Agronomy for Sustainable Development*, *33*(3), 539–549. https://doi.org/10.1007/s13593-013-0135-z

Duru, M., & Ducrocq, H. (1996). A nitrogen and phosphorus herbage nutrient index as a tool for assessing the effect of N and P supply on the dry matter yield of permanent pastures. *Nutrient Cycling in Agroecosystems*, *47*(1), 59–69. https://doi.org/10.1007/bf01985719

EC. (2019). Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulation (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulat. *Official Journal of the European Union*, *2019*(2003), 1–114. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1009&from=EN%0Afile:///C:/Users/gonzalos/Documents/Literature/EC-2019-Regulation(EU)20191009FertilizerProducts.pdf

Florio, A., Felici, B., Migliore, M., Dell'Abate, M. T., & Benedetti, A. (2016). Nitrogen losses, uptake and abundance of ammonia oxidizers in soil under mineral and organo-mineral fertilization regimes. *Journal of the Science of Food and Agriculture*, *96*(7), 2440–2450. https://doi.org/10.1002/jsfa.7364

Frick, H., Oberson, A., Frossard, E., & Bünemann, E. K. (2022). Leached nitrate under fertilised loamy soil originates mainly from mineralisation of soil organic N. *Agriculture, Ecosystems and Environment*, *338*, 108093. https://doi.org/10.1016/j.agee.2022.108093

Guan, P., Wang, R., Nkoh, J. N., Shi, R., Pan, X., Li, J., & Xu, R. (2022). Enriching organic carbon bioavailability can mitigate soil acidification induced by nitrogen fertilization in croplands through microbial nitrogen immobilization. *Soil Science Society of America Journal*, *86*(3), 579–592. https://doi.org/10.1002/saj2.20400

Gwenzi, W., Nyambishi, T. J., Chaukura, N., & Mapope, N. (2018). Synthesis and nutrient release patterns of a biochar-based N–P–K slow-release fertilizer. *International Journal of Environmental Science and Technology*, *15*(2), 405–414. https://doi.org/10.1007/S13762-017-1399-7/FIGURES/6

Joshi, R., Singh, J., & Vig, A. P. (2015). Vermicompost as an effective organic fertilizer and biocontrol agent: Effect on growth, yield and quality of plants. *Reviews in Environmental Science and Biotechnology*, *14*(1), 137–159. https://doi.org/10.1007/s11157-014-9347-1

Kern, J., Tammeorg, P., Shanskiy, M., Sakrabani, R., Knicker, H., Kammann, C., Tuhkanen, E. M., Smidt, G., Prasad, M., Tiilikkala, K., Sohi, S., Gascó, G., Steiner, C., & Glaser, B. (2017). Synergistic use of peat and charred material in growing media–an option to reduce the pressure on peatlands? *Journal of Environmental Engineering and Landscape Management*, *25*(2), 160–174. https://doi.org/10.3846/16486897.2017.1284665

Khiari, L., & Parent, L. E. (2005). Phosphorus transformations in acid light-textured soils treated with dry swine manure. *Canadian Journal of Soil Science*, *85*(1), 75–87. https://doi.org/10.4141/S03-049

Lazcano, C., Gómez-Brandón, M., & Domínguez, J. (2008). Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere*, *72*(7), 1013–1019. https://doi.org/10.1016/j.chemosphere.2008.04.016

Lee, Y. S., & Bartlett, R. J. (1976). Stimulation of plant growth by humic substances. *Soil Science Society of America Journal*, *40*(6), 876–879. https://doi.org/10.2136/sssaj1976.03615995004000060023x

Lim, S. L., Wu, T. Y., Lim, P. N., & Shak, K. P. Y. (2015). The use of vermicompost in organic farming: Overview, effects on soil and economics. *Journal of the Science of Food and Agriculture*, *95*(6), 1143–1156. https://doi.org/10.1002/jsfa.6849

Limwikran, T., Kheoruenromne, I., Suddhiprakarn, A., Prakongkep, N., & Gilkes, R. J. (2018). Dissolution of K, Ca, and P from biochar grains in tropical soils. *Geoderma*, *312*, 139–150. https://doi.org/10.1016/J.GEODERMA.2017.10.022

Liu, M., Wang, C., Liu, X., Lu, Y., & Wang, Y. (2020). Saline-alkali soil applied with vermicompost and humic acid fertilizer improved macroaggregate microstructure to enhance salt leaching and inhibit nitrogen losses. *Applied Soil Ecology*, *156*, 103705. https://doi.org/10.1016/j.apsoil.2020.103705

Luo, W., Qian, L., Liu, W., Zhang, X., Wang, Q., Jiang, H., Cheng, B., Ma, H., & Wu, Z. (2021). A potential Mg-enriched biochar fertilizer: Excellent slow-release performance and release mechanism of nutrients. *Science of the Total Environment*, *768*, 144454. https://doi.org/10.1016/J.SCITOTENV.2020.144454

Mandal, A., Patra, A. K., Singh, D., Swarup, A., & Ebhin Masto, R. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource Technology*, *98*(18), 3585–3592. https://doi.org/10.1016/j.biortech.2006.11.027

Manivannan, S., Balamurugan, M., Parthasarathi, K., Gunasekaran, G., & Ranganathan, L. S. (2009). Effect of vermicompost on soil fertility and crop productivity-beans (*Phaseolus vulgaris*). *Journal of Environmental Biology*, *30*(2), 275–281. http://www.jeb.co.in

Mazeika, R., Dambrauskas, T., Baltakys, K., Mikolajunas, M., Staugaitis, G., Virzonis, D., & Baltrusaitis, J. (2016). Molecular and morphological structure of poultry manure derived organo-mineral fertilizers (OMFs). *ACS Sustainable Chemistry and Engineering*, *4*(9), 4788–4796. https://doi.org/10.1021/acssuschemeng.6b00984

Miao, Y., Stewart, B. A., & Zhang, F. (2011). Long-term experiments for sustainable nutrient management in China. A review. *Agronomy for Sustainable Development*, *31*(2), 397–414. https://doi.org/10.1051/agro/2010034

Morel, J. L., Fardeau, J. C., Béruff, M. A., Guckert, A., Béruff, M. A., & Guckert, A. (1989). Phosphate fixing capacity of soils: A survey, using the isotopic exchange technique, or soils from north-eastern France. *Fertilizer Research*, *19*(2), 103–111. https://doi.org/10.1007/BF01054682

Moritsuka, N., Yanai, J., Mori, K., & Kosaki, T. (2004). Biotic and abiotic processes of nitrogen immobilization in the soil-residue interface. *Soil Biology and Biochemistry*, *36*(7), 1141–1148. https://doi.org/10.1016/J.SOILBIO.2004.02.024

Oberson, A., Tagmann, H. U., Langmeier, M., Dubois, D., Mäder, P., & Frossard, E. (2010). Fresh and residual phosphorus uptake by ryegrass from soils with different fertilization histories. *Plant and Soil*, *334*(1), 391–407. https://doi.org/10.1007/s11104-010-0390-6

Ohno, T., & Zibilske, L. M. (1991). Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal*, *55*(3), 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x

Pare, M. C., Allaire, S. E., Khiari, L., & Nduwamungu, C. (2009). Physical properties of organo-mineral fertilizers. *Canadian Biosystems Engineering /Le Genie Des Biosystems Au Canada*, *51*(1), 21–28.

Parent, L. E., Khiari, L., & Pellerin, A. (2003). The P fertilization of potato: Increasing agronomic efficiency and decreasing environmental risk. *Acta Horticulturae*, *627*, 35–41. https://doi.org/10.17660/ActaHortic.2003.627.3

Parish, F., Sirin, A., Charman, D., Joosten, H., Minayeva, T., Silvius, M., & Stringer, L. (2008). Peatlands and biodiversity. In F. Parish, A. Sirin, D. Charman, H. Joosten, T. Minayeva, M. Silivius, & L. Stringer (Eds.), *Assessment on peatlands, biodiversity and climate change* (pp. 60–98). Global Environment Centre, Kuala Lumpur & Wetlands International.

Qiu, Y., Amirkhani, M., Mayton, H., Chen, Z., & Taylor, A. G. (2020). Biostimulant seed coating treatments to improve cover crop germination and seedling growth. *Agronomy*, *10*(2), 154. https://doi.org/10.3390/AGRONOMY10020154

DIVAPRA, IPLA, & ARPA Regione-Piemonte. (1998). *Metodi di analisi dei compost*. Collana Ambiente.

Regione-Piemonte. (2022). *Allegato I-Fertilizzazione (Par.11 Norme generali-Pratiche Agronomiche)*. Disciplinari di produzione integrata. https://www.regione.piemonte.it/web/temi/agricoltura/agroambiente-meteo-suoli/

Richards, J. E., Daigle, J.-Y., LeBlanc, P., Paulin, R., & Ghanem, I. (1993). Nitrogen availability and nitrate leaching from organo-mineral fertilizers. *Canadian Journal of Soil Science*, *73*(2), 197–208. https://doi.org/10.4141/cjss93-022

Said-Pullicino, D., Cucu, M. A., Sodano, M., Birk, J. J., Glaser, B., & Celi, L. (2014). Nitrogen immobilization in paddy soils as affected by redox conditions and rice straw incorporation. *Geoderma*, *228–229*, 44–53. https://doi.org/10.1016/j.geoderma.2013.06.020

Sakurada, R., Batista, L. M. A., Inoue, T. T., Muniz, A. S., & Pagliar, P. H. (2016). Organomineral phosphate fertilizers: Agronomic efficiency and residual effect on initial corn development. *Agronomy Journal*, *108*(5), 2050–2059. https://doi.org/10.2134/agronj2015.0543

Schmilewski, G. (2008). The role of peat in assuring the quality of growing media. *Mires and Peat*, *3*(2), 1–8. http://www.mires-and-peat.net/

Sica, P., Kopp, C., Müller-Stöver, D. S., & Magid, J. (2023). Acidification and alkalinization pretreatments of biowastes and their effect on P solubility and dynamics when placed in soil. *Journal of Environmental Management*, *333*, 117447. https://doi.org/10.1016/j.jenvman.2023.117447

Singh, B., Pathak, K., Verma, A., Verma, V., & Deka, B. (2011). Effects of vermicompost, fertilizer and mulch on plant growth, nodulation and pod yield of French bean (*Phaseolus vulgaris* L.). *Vegetable Crops Research Bulletin*, *74*(1), 153–165. https://doi.org/10.2478/v10032-011-0013-7

Sørensen, P., Pedersen, B. N., Thomsen, I. K., Eriksen, J., & Christensen, B. T. (2023). Plant availability and leaching of ^{15}N-labelled mineral fertilizer residues retained in agricultural soil for 25 years: A lysimeter study. *Journal of Plant Nutrition and Soil Science*, *186*, 441–450. https://doi.org/10.1002/jpln.202200288

Spohn, M., & Kuzyakov, Y. (2013). Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology and Biochemistry*, *61*, 69–75. https://doi.org/10.1016/J.SOILBIO.2013.02.013

Taparia, T., Hendrix, E., Nijhuis, E., de Boer, W., & van der Wolf, J. (2021). Circular alternatives to peat in growing media: A microbiome perspective. *Journal of Cleaner Production*, *327*, 129375. https://doi.org/10.1016/J.JCLEPRO.2021.129375

Tognetti, C., Mazzarino, M. J., & Laos, F. (2007). Cocomposting biosolids and municipal organic waste: Effects of process management on stabilization and quality. *Biology and Fertility of Soils*, *43*(4), 387–397. https://doi.org/10.1007/s00374-006-0164-8

Traoré, O. Y. A., Kiba, D. I., Bünemann, E. K., & Oberson, A. (2020). Nitrogen and phosphorus uptake from isotope-labeled fertilizers by sorghum and soil microorganisms. *Agrosystems, Geosciences & Environment*, *3*(1), e20111. https://doi.org/10.1002/agg2.20111

Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., & Maggio, A. (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*, *4*(1), Article 5. https://doi.org/10.1186/S40538-017-0089-5/FIGURES/4

Vujinović, T., Zanin, L., Venuti, S., Contin, M., Ceccon, P., Tomasi, N., Pinton, R., Cesco, S., & De Nobili, M. (2020). Biostimulant action of dissolved humic substances from a conventionally and an organically managed soil on nitrate acquisition in maize plants. *Frontiers in Plant Science*, *10*, Article 1652. https://doi.org/10.3389/fpls.2019.01652

Xu, L., & Geelen, D. (2018). Developing biostimulants from agro-food and industrial by-products. *Frontiers in Plant Science*, *871*, 1567. https://doi.org/10.3389/fpls.2018.01567

Zandonadi, D. B., Santos, M. P., Caixeta, L. S., Marinho, E. B., Peres, L. E. P., & Façanha, A. R. (2016). Plant proton pumps as markers of biostimulant action. *Scientia Agricola*, *73*(1), 24–28. https://doi.org/10.1590/0103-9016-2015-0076

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