Conversion from mineral fertilisation to MSW compost use: Nitrogen fertiliser value in continuous maize and test on crop rotation

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Abstract: This study, lasted three years, included results from a continuous maize experiment and from four-year rotation cropping systems (maize, winter wheat, maize and soybean), in which Municipal Solid Waste Compost (MSWC) totally or partially replaced mineral fertilisers. In the first experiment, two different fertilisation strategies, MSWC only (M-Com) and mineral fertilisers (M-Min), were compared with zero nutrients (M-Test 0). Instead, in the rotation cropping systems, two fertilisation practices were matched: mineral fertilisation (R-Min) versus a combination of MSWC and mineral fertilisers (R-Com+Min).

The results, despite a general depression of yields at the beginning of compost application, showed a positive mid-term N fertiliser value of MSWC mainly on yield summer crops and when integrated with N mineral fertilisers. Different soil indicators and the N content in crop tissues and in soil suggested that the scarce N availability of compost was one of the most responsible limiting factors of yield reduction. Due to the very small quantity of MSWC supply, soil total N and the stable organic fraction, intimately bound to mineral phase (MOM), did not vary significantly in the three-year. Conversely, the more labile organic fraction (fPOM) increased only in the top soil layers (0-15 cm). In the top layer, M-Com also increased the amount of organic fraction occluded into soil aggregates (oPOM). Furthermore, compost effectively mitigated N_2O emissions in wheat and maize when replaced N mineral fertiliser. Overall, the fertiliser value of MSWC was maximised when used repeatedly, in combination with mineral fertiliser and especially in spring-summer crops.

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Dear Editor,

Please consider the manuscript “Conversion from mineral fertilisation to MSW compost use: nitrogen fertiliser value in continuous maize and test on crop rotation” for publishing in “SCIENCE OF THE TOTAL ENVIRONMENT”.

We intend to demonstrate that Municipal Solid Waste Compost can replace mineral fertiliser in cereal areas characterized by low availability of other organic fertilisers (manure or sewage sludge), due to its potential as a source of nitrogen and its effectiveness as a tool for soil fertility maintenance.

This study included two experiences, carried out within the same site, located in northwest Italy from 2010 to 2012.

The study demonstrated the positive effects of MSWC on soil N availability, both when used on spring-summer crops and when repeatedly applied. Furthermore, compost effectively mitigated N_2O emissions.

I hope that you will find this paper interesting for a publication in your journal.

Sincerely,

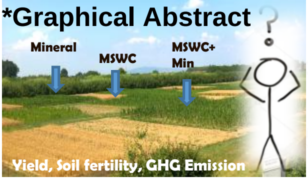
Barbara Moretti

Grugliasco, 20 June 2019

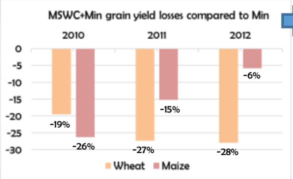
Title:

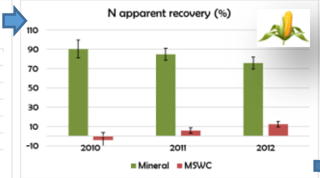
Conversion from mineral fertilisation to MSW compost use: nitrogen fertiliser value in continuous maize and test on crop rotation.

*Graphical Abstract

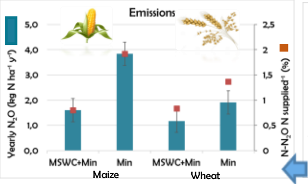


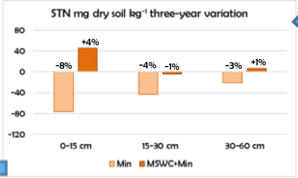
Yield, Soil fertility, GHG Emission





MSWC (+Min):
Better for maize
STN maintenance
Less emissions than Min





***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights:

1. MSWC supports yield performance of spring-summer cereals better than winter cereals.
2. Combination of MSWC with N mineral fertiliser improves Nitrogen Apparent Recovery.
3. MSWC immobilises N in first year of application in minerally-fertilised soils.
4. MSWC increases labile physical organic matter fractions in the top soil layers.
5. MSWC decreases N_2O emissions compared to mineral fertilisation.

Conversion from mineral fertilisation to MSW compost use: nitrogen fertiliser value in continuous maize and test on crop rotation.

Barbara Moretti, Chiara Bertora, Carlo Grignani, Cristina Lerda, Luisella Celi, Dario Sacco

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Abstract

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Overall, the fertiliser value of MSWC was maximised when used repeatedly, in combination with mineral fertiliser and especially in spring-summer crops.

Keywords:

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1. Introduction

In stockless areas, where organic fertilisers are in low availability, the use of Municipal Solid Waste Compost (MSWC) is a valuable alternative to manure or sewage sludge (Ros et al., 2006). MSWC is considered a potential source of nutrients for plants (Bar-Tal et al., 2004; Weber et al., 2007), mainly nitrogen (N) (Erhart et al., 2005; Soumaré et al., 2003; Warman et al., 2009), and an effective tool for soil organic matter (SOM) restoration (Alluvione et al., 2013; Annabi et al., 2007; Chalhoub et al., 2013; Montemurro et al., 2006; Weber et al., 2007).

However, according to literature, when applied alone, the first years of MSWC use show yields, grain protein content or straw N content reduction with respect to mineral fertilisation treatments (Alluvione et al., 2013; Erhart et al., 2005; Martínez-Blanco et al., 2013; Montemurro et al., 2006; Weber et al., 2014), due to lower N availability for the crop. Especially during conversion from mineral fertiliser management to MSWC, the plant-soil system cannot indeed rely on cumulative residual N effects from past applications, as usually happens in long term organically fertilised systems (Monaco et al., 2010). Alluvione et al. (2013) found this initial yield reduction even in a pedo-climate with an estimated SOM mineralisation coefficient of about 2%, conceptually guaranteeing a good availability of mineral forms (Bertora et al., 2009).

The early reduction in N availability can be at least partially counteracted in the mid-term (two to 10 years) (Martínez-Blanco et al., 2013), benefitting from repeated applications, which contribute to both select efficient microbial communities for degradation and mineralisation (García-Gil et al., 2000) and build an organic stock of nutrients (Monaco et al., 2010). Some strategies can mitigate this early yield loss. The combination of MSWC with N mineral fertilisers, for instance, certainly improves the early N availability for crop, not only because of the presence of N in promptly available forms, but also due to the stimulation that the added mineral N exerts on the soil microbial community (Bhattacharyya et al., 2005; Busby et al., 2007; Montemurro et al., 2006).

Furthermore, N immobilisation via abiotic reactions of N inorganic forms with the lignin component of compost may also lead to stable N-containing compounds (Knicker et al., 1997; Olk et al. 2006; Schmidt-Rohr et al., 2004), which further reduces both N mineral concentration in soil solution and N feedback to microbial utilisation. Aside from the equilibrium between biotic and abiotic N immobilisation/mineralisation, the spatial distribution of compost components into aggregates and their physical and chemical interactions with finer soil particles (Six et al., 2002) can further affect N cycling and its availability for crops (Said-Pullicino et al., 2014).

Chemical-biochemical properties of compost itself influence significantly N mineralisation activity (Busby et al., 2007; Chalhoub et al., 2013; Gilmour et al., 2003; Weber et al., 2014), and leads to soil microbial community specialisation (Iovieno et al., 2009; Weber et al., 2014). Compost characterised by a low C/N ratio generally increases soil N availability, even in the short term (Gale et al., 2006; Gilmour and Skinner, 1999; Weber et al., 2014) due to a prevailing net N mineralisation. In other cases, compost may cause an initial N immobilisation (Erhart et al., 2005; Fiorentino et al., 2016; Hargreaves et al., 2008). This phenomenon usually occurs when the applied compost has a high C/N ratio (>20), which reduces N availability in the short term (Chalhoub et al., 2013; Gale et al, 2006) and increases the pool bound to soil organic matter (SOM), i.e. soil organic N (SON). Repeated applications of MSWC may lead to enhanced N bioavailability later through higher contributions of soluble inorganic N forms coming from microbial biomass and SON mineralisation (Chalhoub et al., 2013; Diacono and Montemurro, 2010; Monaco et al., 2008). Moreover, also the presence of highly-stabilised organic matter components can have lower N mineralisable potentials if compared to low C/N and low-stabilised compounds (Gilmour et al., 2003).

Furthermore, as with other organic fertilisers, the ability of compost to provide N to crop depends also on the synchronisation of mineral N availability and N requirements by plants, otherwise limiting N uptake and crop yield (Erhart et al., 2005, Evanylo et al., 2008, Montemurro et al., 2006). Synchronisation between N availability and N uptake is pivotal not only for improving N use efficiency, but also for minimising N losses. Sacco et al. (2015) showed similar potential N losses in cropping systems managed with composted organic fertilisers and mineral fertilisers (76 and 101 kg ha^{-1} year^{-1}, respectively) while Alluvione et al. (2010) found that MSWC application reduced N_2O emissions (0.11% of applied N) compared to urea (3.4% of applied N).

Based on all these considerations, it is clear that MSWC application should be carefully managed to optimise N uptake and use efficiency while limiting potential N losses. This work is aimed to evaluate the effect of a three-year application of MSWC characterised by low C/N ratio and highly-stabilised organic compounds in a continuous maize cropping system. The evaluation is addressed to assess the MSWC N fertiliser value, i.e. its ability to sustain the N requirement of maize for grain production and to enhance soil N availability. Then the results are used to interpret the application of MSWC in combination with mineral fertilisers in a four-year rotation system typical of grain commercial farms of the area. The combination of the two experiences was aimed to identify the most appropriate MSWC application methods in terms of crop production and N efficiency while limiting the environmental impact due to N losses from the agro-ecosystem.

2. Materials and methods

2.1 Site description

The present study is based on a continuous maize experiment (MAIZE experiment), and on a four-year crop rotation trial (ROT trial). Both were located in the western Po River plain (NW Italy) in a 1.3 ha experimental platform (Lombriasco: 44° 50' 39" N, 7° 38' 15" E; 241 m a.s.l.) from 2010 to 2012.

According to Köppen-Geiger classification, the climate of the area is Cfa (Kottek et al., 2006) with a mean annual temperature (from 2010 to 2012) of +12.5°C and an average minimum and maximum monthly temperature of +9.1 and +19.1°C. The mean annual precipitation of the same period was 846 mm with two main rainy periods during April-May and October-November. Figure 1 reports the main climatic parameters of the 2010-2012 period, compared to longer climatic series.

The soil was classified as loamy sand, coarse-loamy mixed non-acid mesic Typic Hapludalf (USDA, 2010). The ploughed horizon (0-30 cm) contained 52 and 9% of sand and clay, respectively, and was characterized by neutral pH (6.8). Soil organic carbon (SOC) was 8.9 g kg^{-1} and C/N ratio was 9.0. Cation exchange capacity was 9.42 cmol$_{(+)}$ kg^{-1}, exchangeable K 67 mg kg^{-1} and available P (P Olsen) was 67 mg kg^{-1}.

2.2 MAIZE experiment

2.2.1 Design and agronomic management

The field experiment (MAIZE, M-) was based on continuous maize (*Zea mays* L.) for grain production and lasted three years. Before the onset of the experiment, the soil that never received compost, was cropped with maize for grain and managed homogenously. Crop residues were always left and incorporated into soil.

Three treatments, placed on plots of 4.2 m wide and 10.0 m long (42.0 m^2), were set up in a randomised complete block design with four replicates. The treatments varied in fertilisation management as follows:

i) *M-Test 0*: Zero N, P, and K fertilisation, used as a control treatment;

ii) *M-Com*: MSWC supplied at average rate of 8.8 Mg of dry matter (DM) ha^{-1} year^{-1};

iii) *M-Min*: Total N, P_2O_5 and K_2O, supplied as mineral fertilisers (urea 46%, potassium chloride 60%, and triple superphosphate 46%) and used for comparison.

The amount of nutrients supplied in the treatments was equal for both *M-Com* and *M-Min* (on average 198, 98 and 126 kg ha^{-1} year^{-1} for N, P_2O_5 and K_2O respectively). The amount of MSWC supplied in *M-Com* was set considering the N threshold indicated for maize grain production (200 kg ha^{-1} year^{-1}) in Integrated Agriculture Techniques (IAT) of Regional Rural Development Programmes (EC No 1698/2005) and the actual N content of compost, measured every year before spreading. The amount of P_2O_5 and K_2O resulted from N/P_2O_5 and N/K_2O ratio specific for MSWC applied. In *M-Min* P and K mineral fertilisers supply varied annually to achieve *M-Com*.

All treatments were mouldboard ploughed at 30 cm depth in autumn, to incorporate plant residues of the previous year, and then disk harrowed twice in spring two days before seeding. All fertilisers, either MSWC or minerals, were spread in spring before maize seeding and between the two disk harrowing at the same time. Maize hybrid “SNK Famoso” (FAO rating 500; 127 days) was seeded at a density of 7.1 plants/m^2. Seeding and harvesting dates were the same reported for maize of crop rotation trial (Table 2). Drip irrigation supplied 40-60 mm of water two - four times yearly, as needed. Only one post-emergence herbicide was applied at four leaves unfolded maize growth stage.

2.2.2 Compost characteristics

MSWC was produced by mixing green wastes (park and garden residues derived from pruning) and organic domestic wastes as defined by Mainero (2008). Compost samples were collected and characterised. Most chemical parameters varied widely (Table 1); only the pH value remained alkaline. Total N content was similar to that of bovine farmyard manure common in the area (Grignani et al., 2007) and this is an interesting characteristic for an eventual replacement. The C/N ratio was 9.6. The fibre fraction contents (NDF, ADF, and ADL) were quite stable thanks to composting and maturing processes (Busby et al., 2007); ADL (lignin fraction) represented 48% of NDF (total fibre). However, N content of NDF varied widely and accounted for 20% of total N. $N\text{-}NH_4^+$ was relatively low (0.4 g kg^{-1}, corresponding to 2% of total N) and with high variability (78% CV coefficient). Soluble N, calculated subtracting N-NDF from total N, accounted for 80% of total N and was almost constant among the different samples. Because of the low value of $N\text{-}NH_4^+$, soluble N was represented mostly by organic forms.

2.2.3 Crop yields, N content and estimation of maize N availability indicators

For measuring yield and N concentration, maize grain and straw were manually collected at physiological maturity from a total area of 12 m^2 (4 m x 2 rows with 0.75 m inter-row spacing x two sampling areas) in each plot. Plant samples were oven-dried at 60°C for 72 hours and the dry matter subsequently determined. Grain and straw total N was analysed by means of a CN elemental analyser (Flash EA 1112, Thermoquest, MIPAF method, 2000, Italy).

Nitrogen Apparent Recovery (NAR) for mineral and for MSWC treatments ($NAR_{M\text{-}Min}$ or $NAR_{M\text{-}Com}$) was estimated using equation 1 (Zavattaro et al., 2012):

$$NAR = \{[(Y*N)_{\text{Grain}} + (Y*N)_{\text{Residue}}]_{M\text{-}Min\ \text{or}\ M\text{-}Com} - [(Y*N)_{\text{Grain}} + (Y*N)_{\text{Residue}}]_{M\text{-}Test\ 0}\} / \text{F}c\ \text{or}\ \text{F}o \quad (1)$$

where Y is the maize grain and residue yield and N is the corresponding N tissue content (i.e. N uptake of grain or residue) of *M-Min*, *M-Com*, or *M-Test 0*; F*c* is the total N input (kg ha^{-1}) from mineral fertilisers; F*o* is the total N input (kg ha^{-1}) from MSWC.

The second indicator of N availability was the Nitrogen Fertiliser Replacement Value (NFRV), estimated as the $NAR_{M\text{-}Com}$ to $NAR_{M\text{-}Min}$ ratio.

2.2.4 Determination of soil N forms

In MAIZE experiment treatments, soil was sampled at the beginning (October 2009) and at the end (October 2012, after maize harvesting) of the three-year period. Three soil samples were randomly collected with a hand corer at three depths (0-15, 15-30, and 30-60 cm) from each plot and pooled into a composite sample. The composite samples were then air-dried and sieved at 2 mm.

Soil total N (STN) was measured as described for crop tissues above. Soil OM fractionation was performed on the samples collected in October 2012 as described by Golchin et al. (1994) and modified by Cerli et al. (2012). Briefly, 125 ml of Na-polytungstate (NaPT) solution (density 1.6 g cm^{-3}) was added to 25 g of soil. The suspension was gently hand shaken to ensure complete soil wetting and to avoid aggregate disruption, then allowed to settle for 1 h. After centrifugation at 5600 rpm for 20 min, the light fraction (free particulate organic matter, *f*POM) was collected on a 0.7-μm glass microfibre filter, rinsed with deionized water, air dried, and finely ground. The remaining soil was suspended again in fresh NaPT solution and treated ultrasonically (275 J ml^{-1}). After centrifugation, the light fraction released from aggregate breakdown (occluded particulate organic matter, *o*POM) was separated, washed, dried, and finely ground as described above. Preliminary tests were performed to select the appropriate sonication energy to release the entire pool of *o*POM, while avoiding contamination by minerals and the release of organic-mineral complexes (Cerli et al., 2012). The remaining soil (mineral-associated organic matter, MOM) was washed with deionized water until salt-free, then dried, and ground. Mass yields, as well as organic C and N contents, were determined on all fractions.

2.3 ROT trial

2.3.1 Design and agronomic management

Analyses performed in the MAIZE experiment were extended to a trial with crop rotation system (ROT, R-) similar to grain commercial farms of the Po river plain. ROT trial was organised as a randomised complete block design and placed close to MAIZE experiment during the same period. Therefore, climate and soil characteristics were the same of the first experimental trial. It compared two cropping systems, characterised by four-year rotation of winter wheat (*Triticum aestivum* L., “Bologna” variety), maize for grain (*Zea mays* L., seeding the same hybrid of MAIZE experiment), soybean (*Glycine max* L., “PR91M10” variety, maturity group 0+, reseeded in 2011 with “Brillante” variety, maturity group 1-), and then maize for grain again. Each cropping system included four plots of 12.6 m wide and 26.0 m long (328.0 m^2 each), managed with standard farm machinery, in which the four crops were hosted simultaneously every year.

Both cropping systems were mouldboard ploughed to 30 cm in autumn and then disk harrowed twice two days before seeding in autumn for winter wheat (236 kg ha^{-1}) and in spring for maize and soybean (7.1 and 50 plants m^{-2} respectively). In table 2 was reported seeding and harvesting date for all crops.

Similarly to MAIZE experiment, the fertilisation of the two cropping systems was managed according to IAT as defined by the regional Rural Development Programmes, and distinguished:

i) *R-Min*: managed with mineral fertilisers as control;

ii) *R-Com+Min*: managed with a combination of MSWC (the same used in MAIZE Experiment) and mineral N fertilisers.

Table 2 reports the amount of N, P_2O_5 and K_2O supplied as MSWC or mineral.

In *R-Min*, maize received 20% of urea (40 kg N ha^{-1} $year^{-1}$) during seeding and the remaining 80% (160 kg N ha^{-1} $year^{-1}$) was split in top dressings between two tranches: at the eight leaf unfolded and at the second detectable node. Winter wheat was top-dressed in spring with ammonium nitrate (27% N) in two rates: at the beginning of tillering (60 kg N ha^{-1} $year^{-1}$) and at the beginning of stem elongation when the first node was at least one cm above tillering node (80 kg N ha^{-1} $year^{-1}$). No N fertilisation was supplied to soybean. No P fertiliser applications were needed for any crop as the soil available P content was above the threshold (P_{Olsen}>20 mg kg^{-1}) set by IAT fertilisation management. On the other hand, potassium chloride was distributed in a single application after the first disk harrowing in autumn for winter wheat, and in spring for maize and soybean.

In *R-Com+Min*, MSWC was distributed and incorporated before the second disk harrowing in autumn for winter wheat, and in spring for maize and soybean at a rate of 4.3, 6.6 and 3.0 Mg DM ha^{-1} $year^{-1}$, respectively. In addition to MSWC, 50 kg N ha^{-1} of mineral fertiliser was applied at top-dressing as ammonium nitrate for winter wheat at the beginning of tillering stage, and as urea for maize at eight leaf unfolded stage. Consequently, N supplied via mineral fertilisers in wheat and maize represented 37 and 25% of the total N applied on average for the three-year period, respectively. No N mineral fertiliser was supplied to soybean. No P mineral integration was required as MSWC contained sufficient P for all crops. Potassium chloride was supplied simultaneously with MSWC, before the second disk harrowing, in autumn for winter wheat and in spring for soybean (93 and 26 kg K_2O ha^{-1} year^{-1}, respectively). No K mineral integration was necessary for maize as the high amount of MSWC supplied and the balanced N/K_2O ratio was adequate to maize uptake.

In both cropping systems, crop residue was left in the field and incorporated into the soil except for winter wheat, where it was removed following the typically local management. Finally, for maize and winter wheat, only post-emergence herbicides were applied; for soybean, pre- and post-emergence operations were necessary. Drip irrigation supplied to maize and soybean 40-60 mm of water for each irrigation event 2-4 times year^{-1}, as needed.

2.3.2 Crop yield, N content and estimation of maize N availability indicator

In the ROT trial, maize grain and straw were sampled from an area of 18 m^2 (4 m x 3 rows with 0.75 m inter-row spacing x two sampling areas) in each plot and collected as described for MAIZE experiment. Grain and straw of winter wheat and soybean were mechanically collected by means of a plot combine harvester from a total area of 18 m^2 (6 m length x 1.5 m width x two sampling areas) in each plot.

Maize NAR was estimated for *R-Min* ($NAR_{R\text{-}Min}$) and for *R-Com+Min* ($NAR_{R\text{-}Com+Min}$). Total N uptake of maize in the two cropping systems and the total N input (kg ha^{-1}) spread with N mineral fertilisers or MSWC plus N mineral fertilisers were used, as described in equation (1). Lacking in this trial a zero N treatment, *M-Test 0* was considered for the calculation.

2.3.3 Determination of soil N forms

Also for ROT trial, soil was analysed for STN (in October 2009 and October 2012) and SOM fractionation (in October 2012). Methods and periods of sample collection as well as the methods for chemical analysis were the same described for MAIZE experiment.

Soil nitrate ($N\text{-}NO_3$) content was determined by collecting samples from the first layer (0-30cm) in March, May, July, and October during the three cropping seasons (2010-2012) for each crop of both cropping systems. Nitrate was extracted from soil with 1 M KCl for 1 h and determined by colorimetry with a continuous flow analyser (Evolution II, Alliance Analytical Inc., Menlo Park, CA).

Soil Potential Mineralisable Nitrogen (PMN) was measured after crop harvesting, to estimate the availability of labile organic N remained in the soil at the end of the three years of two different fertilisation management techniques. Soil samples were collected at the same time of STN sampling (October 2012) from the 0-30 cm layers and incubated anaerobically for 30 days (Keeney, 1982). PMN measurements were limited to maize and winter wheat crop.

2.3.4 Nitrous oxide emissions

A further analysis was carried out from October 2010 to January 2012 within ROT trial to estimate N_2O emissions from the *R-Min* and *R-Com+Min* cropping systems, in the plots cropped with maize and wheat. Emissions were measured applying a non-steady-state closed chamber technique (Livingston and Hutchinson, 1995) with four replicates per cropping system. Stainless steel anchors (75x36 cm for maize and 36x27 cm for wheat) were inserted 15 cm into the soil and remained there throughout the experimental period, except for the period between tilling and seeding during which they were removed for soil management. Wooden boards were adopted to access the anchors during sampling to avoid soil compaction or crop disturbance. During each measurement event, a rectangular stainless steel chamber (75x 36 x20 cm high for maize and 36x27x20 cm high for wheat) was sealed to each anchor by means of a water-filled channel. Plants were never included in the chamber headspace. Headspace gas samples internal headspace were collected by propylene syringes at 0, 15, and 30 min after chamber closure. Thirty-milliliter air samples were then injected into 12-mL evacuated vials, closed with butyl rubber septa (Exetainer1 vial from Labco Limited, UK). Gas concentrations of the samples were determined by gas chromatography with a fully automated gas chromatograph (Agilent 7890A), equipped with electron capture detector for N_2O quantification. Fluxes were calculated from the linear or nonlinear (Hutchinson and Mosier, 1981) increase in concentration (selected according to the emission pattern) in the chamber headspace over time (Livingston and Hutchinson, 1995). Estimates of cumulative emissions for each plot were based on linear interpolation across sampling days (Bertora et al., 2018).

2.4 Statistical analysis

Data were analysed using a two-ways ANOVA, separately for MAIZE and ROT studies. Results were first checked for normal distribution and homoscedasticity. Statistical analysis for yields and N tissue concentration tested treatment or system (for MAIZE experiment or ROT trial, respectively), year and interaction effects.

Soil parameters were analysed through a one-way ANOVA when not replicated over the period (SON fractionation and PMN), checking only treatment or system effects. When measurements were repeated on the same statistical units at the beginning and end of the experiment (STN), a repeated measure model was applied.

Yearly cumulative N_2O emissions were analysed for wheat and maize of both ROT cropping systems (*R-Min* and *R-Com+Min*) and ANOVA model included crop and system effects.

Block effect was included in the analysis for agronomical measurements, but not for soil chemical characterisation.

Pearson correlation was used to estimate the relationship between N content and respective grain and straw yield for all of the cropping systems and treatments of both the ROT and MAIZE studies; the results were pooled from the three-year period.

3. Results

3.1 MAIZE experiment

3.1.1 Crop yields and tissues N content

Fertilisation management affected maize grain and straw yields (Table 3).

In terms of grain yield, *M-Min* was always the most productive. The *M-Com* treatment produced the same as *M-Test 0* for the first two years. However, a significant interaction (treatment * year) was present, as during the third year *M-Com* was the intermediate treatment and yielded 55% more than *M-Test 0.* The *M-Min* treatment was the most productive also for plant residue. The same trend of progressive increase of the initial difference between *M-Com* and *M-Test* was observed, but the two treatments never differed significantly. Year effect revealed 2010 as the most productive for both grain and straw. These high productions were attributed to the unusually high amount of rainfall during the 2010 April-October growing period (581 mm, 267 mm, and 417 mm respectively in 2010, 2011, and 2012) if compared to the same period from 1925 to 2012 (Figure 1).

Grain and residue N concentrations did not reveal any interaction effect between treatment and year. *M-Min* was always the highest and *M-Com* never differed from *M-Test 0.* Year 2010 showed the highest concentrations.

3.1.2 Maize N apparent recovery

As shown in Figure 2, N apparent recovery of *M-Min* ($NAR_{M\text{-}Min}$) reduced progressively from 2010 to 2012 (90, 85 and 76% respectively). This was because of the progressive reduction of the difference between *M-Min* and *M-Test 0* total N uptake (grain + straw) (179, 170, 151 kg ha^{-1} for 2010, 2011 and 2012, respectively). Conversely, the $NAR_{M\text{-}Com}$ values progressively increased from 2010 to 2012, due to a gradual increase of *M-Com* plant uptake.

Consequently, N Fertiliser Replacement Value (NFRV), increased gradually from 2010 to 2012: 5, 7, and 16% for 2010, 2011, and 2012, respectively.

3.1.3 Soil total N and SON fractions

Values of STN did not show variation between treatments from 2009 to 2012 (Table 4).

Years induced a significant STN reduction from 2009 to 2012 in the first and in the third layers, independently of fertilisation management. On the contrary, 2012 SON fractions in the first layer revealed significant differences: N content in *f*POM and *o*POM were higher in *M-Com* than in *M-Test 0* and *M-Min,* whereas the MOM fraction was unaffected by treatments. *M-Test 0* and *M-Min* revealed the same values of SON fractions between different layers.

3.2 ROT trial

3.2.1 Crop yields and tissues N content

Maize grain yield revealed highly significant interaction effect of system by year (Table 5). This derived from a progressive yield improvement of *R-Com+Min* over the years showing reductions of 26% (first year), 15% (second year), and no significant difference (third year) with respect to *R-Min* grain yield. In maize straw, no statistical effect was detected, except for year, revealing 2010 as the most productive for both cropping systems.

While winter wheat yields were significantly affected by both cropping system and year, for neither grain nor straw yields interaction effect was significant. *R-Com+Min* grain and straw always produced less than *R-Min*. On average, *R-Com+Min* yields were reduced 33 and 19% for grain and straw with respect to *R-Min.* The higher rainfall occurred during the tillering stage in 2010 and 2011 (Figure 1) and the seeding delay in the same years (Table 3) induced the rank of yield by year 2012>2010>2011 for grain and straw. The two effects reduced shoot density in both systems as shown by the mean values of 610 and 537 shoots/m^2 in 2010 and 2011, respectively, *versus* 899 shoots/m^2 in 2012 for the two cropping systems.

Soybean grain yield showed no significant effects of cropping system, year or interaction between factors. Only straw yield resulted significantly lower in 2011 when soybean was necessarily re-seeded using a variety belonging to maturity group 1(-), characterised by a slower maturity cycle than the customary group 0(+) (Table 2).

The N content of maize grain and residue (Table 6) was always higher in *R-Min* than in *R-Com+Min,* and no interaction was detected.

The N content of winter wheat tissues indicated a significant effect of the interaction system*year both for grain and straw. Although *R-Com+Min* showed values consistently lower than *R-Min*, the gap between the two cropping systems was reduced over the three-year period. Indeed, across 2010, 2011, and 2012, the N content in *R-Com+Mi*n was 59, 33, and 32% lower for grain and 63, 37, and 32% lower for straw than in *R-Min*, respectively.

N concentration in soybean never displayed differences by systems or in interaction with year.

At last, straw N content highlighted a significant year effect for all crops. Maize straw N concentration was 31% higher in 2010 than in the other two years, 38% for winter wheat and 63% for soybean, a result likely due to abundant rain that promoted grain and straw yield and N concentration in continuous maize experiment.

Nitrogen apparent recovery (NAR) of maize cropped in *R-Com+Min*, measured considering *M-Test 0* to estimate N natural availability for maize over the three-year period, showed an increase from 2010 to 2012 as follows: 34, 50 and 51%. Similar to $NAR_{M\text{-}Min}$, $NAR_{R\text{-}Min}$ showed a progressive decrease across the three-year: 91%, 88% and 82% respectively for 2010, 2011 and 2012.

3.2.2 Soil total N, SON fractions, and nitrates

Soil total N differed between the two cropping systems only in the first layer (0-15 cm) but without a system*year interaction (Table 7). This result indicated that the differences existing before the onset of the experiment remain similar at the end of the experimental period. Therefore, MSWC application did not induce variation N stocked in soil. In the same way, the C/N ratio never showed significant differences.

Similar to MAIZE experiment, N in *f*POM varied between the two cropping systems. *R-Com+Min* was higher than *R-Min,* whereas N content in *o*POM and MOM were constant between the two systems.

Soil nitrate content measured for both cropping systems in the 0-30 cm layer (Figure 3) showed a different behaviour among years and crops. In general, for all crops and cropping systems, values were usually lower in March than in the previous October. With the exception of the peak measured in July 2012 in soybean, values for the three crops were higher in 2011 than in the other two years, corresponding to low rainfall from May to October (Figure 1). Comparing the three crops, the greatest values were obtained with maize and soybean in late spring or summer (May-July), when high temperatures promoted mineralisation activity. Furthermore, in soybean, higher values were detected than in maize, as N fixation and low crop yield reduced crop uptake (92 kg N $ha^{-1}year^{-1}$ in soybean, after deduction of N derived from fixation, versus 202 kg N ha^{-1} $year^{-1}$ in maize). On the contrary, the absence of winter wheat crop uptake after the July harvest and the high mineralisation of SOM and limited rainfall events caused the greatest values in autumn.

The comparison between cropping systems showed no differences, considering the average of all collected data (6.9 e 7.2 mg kg^{-1} dry soil for *R-Min* and *R-Com+Min*, respectively). When considering values of individual months (i.e 36 months for data collection), differences were instead important: *R-Min* overcame *R-Com+Min* seven times out of 36, while *R-Com+Min* overcame *R-Min* five times out of 36.

Potential Mineralisable N (PMN), measured in plots hosted maize and winter wheat for both cropping systems at the end of the study, revealed higher values in maize than in winter wheat (Table 8). In addition, PMN showed an important system effect; PMN in *R-Com+Min* was 46% higher than in *R-Min*. The PMN: STN ratio showed the same trend, highlighted by a 43% higher value in *R-Com+Min* than in *R-Min*.

3.2. Nitrous oxide emissions

Nitrous oxide (N_2O) emission measurements covered both the wheat and maize cropping cycles and intercropping periods in the ROT trial for a total of 15 months (Figure 4). The winter wheat firstly peaked in November, a few weeks after seeding, earlier for *R-Com+Min* (0.019 kg N ha^{-1} d^{-1}) than for *R-Min* (0.015 kg N ha^{-1} d^{-1}, one week later). An analogous intensification of fluxes was observed in the subsequent autumn (November 2011) when peaks in the two cropping systems occurred concurrently (0.029 and 0.026 kg N ha^{-1} d^{-1} for *R-Com+Min and R-Min*, respectively). Shortly after vernalisation (at the beginning of tillering stage), the first top-dressing fertilisation triggered emissions that produced a minor peak (0.010 and 0.009 kg N ha^{-1} d^{-1} for *R-Min* and *R-Com+Min*, respectively). A more intense peak was subsequently observed only for *R-Min* (0.023 kg N ha^{-1} d^{-1}). The highest peak was reached at the beginning of stem elongation, just few days after the last top-dressing N fertilisation, more intense in *R-Min* than in *Com+Min* (0.051 and 0.012 kg N ha^{-1} d^{-1}, respectively).

Emission dynamics in maize started after pre-seeding fertilisation and reached a first peak approximately three weeks later (0.017 and 0.018 kg N ha^{-1} d^{-1} in *R-Min* and *R-Com+Min*, respectively). The major peak was reached at the eight leaf unfolded, approximately two weeks after the first top-dressing fertilisation, and corresponded to 0.302 and 0.077 kg N ha^{-1} d^{-1} in *R-Min* and *R-Com+Min*, respectively. No peak was detected at the second top dressing N mineral fertilisation neither for *R-Min* nor for *R-Com+Min* that received 50 N mineral kg ha^{-1}. In both cropping systems, instead, a minor peak was observed (0.023 kg N ha^{-1} d^{-1} on average) in late autumn on bare soil, a few weeks after ploughing, both in 2010 and 2011.

Yearly cumulative fluxes (from November 2010 to November 2011) were significantly affected by both crop and system, with the highest values induced by *R-Min* on maize (3.84 kg N ha^{-1} y^{-1}) and lowest by *R-Com+Min* on wheat (1.18 kg N ha^{-1} y^{-1}) (Figure 5). Emission factors expressed as kg N-N_2O lost/kg N applied and calculated by subtracting the background N_2O emitted from an unfertilised plot set apart for this use, were on maize 1.93 and 0.23 for *R-Min* and *R-Com+Min*, and on wheat 0.96 and negative for *R-Min* and *R-Com+Min*, respectively.

4. Discussion

4.1 N availability for crop nutrition

Both grain and residue maize yields in *M-Com* (continuous maize experiment fertilised with MSWC alone) were similar to treatment with no fertilisers supply. Since in *M-Com* the mean P content of maize tissues resulted higher than in *M-Min* (3.1 and 2.0 g kg^{-1} DM, respectively) and K was similar (8.5 and 8.9 g kg^{-1} DM, respectively), the N availability appeared to be the main limiting factor of the maize yield reduction. To emphasise this, N content in *M-Com* grain and residue was always lower than *M-Min*, and similar to *M-Test 0*. As a consequence, *M-Com* NAR (5% average for three years) was always considerably lower than *M-Min* (84%) and even negative in the first year of MSWC application.

Different authors confirmed the scarce concentration of N available forms for plants in treatment managed with only MSWC and an initial N immobilisation due to increase of microbial biomass and activity (Alluvione et al., 2013; Erhart et al., 2005; Evanylo et al., 2008; Fiorentino et al., 2016; Hargreaves et al., 2008).

However, the progressive increase of *M-Com* NAR and NFRV during the three-year period indicated an improvement of soil available N forms suitable to meet crop requirements. A cumulative residual N effect probably derived from the continuous applications of MSWC, affecting positively N content in crop tissues and grain yield in *M-Com* during the last year. This is in agreement with Monaco et al. (2010), who demonstrated that in a soil treated with organic fertiliser for several years, the N available to crop derived from the previously built soil fertility rather than from the organic fertiliser provided in the year. This is reinforced by the fact that 98% of MSWC N was represented by stable organic compounds that can easily store in soil. Moreover, *M-Com* NFRV achieved 16% in 2012 (compared to only 5% in 2010) suggesting a gradual increase of compost potentiality to substitute N mineral fertilisation for maize. This hypothesis may be applied also to the four-year rotation cropping systems.

The low N availability of MSWC and its effect on the yield reductions can be indeed assumed also in ROT trial, because as reported for MAIZE experiment, P and K elements content in tissues of all crops never resulted lower than mineral fertilised cropping system (data not shown).

However, MSWC affected differently crop productions. Except for soybean (N-fixing crop), in which N content in grain and straw never revealed differences between systems managed with compost+mineral or mineral fertilisation alone, the average of N content in winter wheat grain and straw was 29 and 31% lower in *R-Com+Min* than in *R-Min* respectively, and it was 22 and 28% lower in maize. Consequently, N concentration in crop tissues could be rank as follows: soybean > maize > winter wheat.

Yield results followed the same rank of N concentration. The three-year period grain yield and straw average, showed no differences between *R-Com+Min* and *R-Min* for soybean. Instead, a grain reduction of *R-Com+Min* of 16% in maize and 24% in winter wheat was measured. Straw/residue yields behaved similarly (-6% and -16%, respectively). Furthermore, this is in agreement with Weber et al. (2014) who pointed out that winter cereals are particularly penalised by MSWC fertilisation in terms of production.

As expected, MSWC behaved as any other composted organic fertilisers, like manure or commercial organic fertilisers (poultry manure, manure, feather meal, and oleaginous residues mixed) (Sacco et al., 2015). The most available N fraction ($N\text{-}NH_4^+$) in MSWC, indeed, accounted only for about 2% of total N. The remaining N potentially plant absorbable pool probably derived from the decomposition and mineralisation of the soluble organic fraction (80% of total N), which decomposes faster than other organic components. Garnier et al. (2003) reported a constant decomposition rate of 1.49 d^{-1} for the soluble organic fraction *vs* 0.10 d^{-1} for hemicelluloses, 0.15 d^{-1} for cellulose, $2.22 \cdot 10^{-3}$ d^{-1} for lignin, and $0.126 \cdot 10^{-3}$ d^{-1} for humified organic matter. Therefore, grain yield, together with N content in crop tissues, indicated that the application of compost induces and take advantages of microbial activities of decomposition and mineralisation that are more intense and useful during the maize growing period (late spring and summer) than during winter wheat growing period (winter and spring). Thanks to a more intense N mineralisation and longer synchronisation between N release and crop uptake, maize benefitted more from MSWC fertilisation than winter wheat, especially after three years of application. In winter wheat, low temperature at tillering growth stage (i.e. during January-February) led to a reduced available N pool, inducing a low stem density and consequently a low grain yield. The N fertiliser value of MSWC was thus unequal between crops, better performing on summer than on winter crops.

In this study, a progressive increase of MSWC for N availability in the three-year period could be detected for three reasons. First, a continuous rise of grain yield during three-year period was detected in *R-Com+Min* for maize reaching in the last year the productive level of *R-Min.* Second, a gradual growth of maize NAR from the first to the third year both for *M-Com* and *R-Com+Min* (+16 and +17%, respectively). Third, the gap of N content winter wheat tissues between compost+mineral and mineral management, was reduced from -37 to -24% for grain and from -39 to -24% for straw from the first to the third year of the study. The N content of maize tissues did not increase following the repeated addition of MSWC, neither in the experiment nor in rotation cropping systems, because it is a high N-demanding crop (Zavattaro et al., 2012) and MSWC applied in this study was unable to fully balance N maize requirements.

The insufficient N availability for maize is highlighted also by the significant correlation found between N content in maize grain and straw and their respective yields (Figure 6), both in continuous system or in crop rotation. It is possible to rank treatments on available N for plants as follows: *M-Test 0 = M-Com < R-Com+Min < M-Min = R-Min.* Mineral fertilisation (*M-Min* and *R-Min*) producing the highest yields with the high N content in crop tissues, while *R-Com+Min* gave intermediate values. Conversely, the application of MSWC alone in maize produced the lowest yields and N content as no fertilised control.

These results confirm that MSWC furnished better results for maize, if applied in combination with mineral prompt fertilisers. Moreover, aside from the presence of microorganisms specialised to organic material decomposition (due to previous management of ROT *Com+Min* plots), N mineral supplement promotes mineralisation of the soluble organic fraction that enhances the availability of this N source (Garnier et al., 2003), and consequently increase crop yield (Bhattacharyya et al., 2005; Busby et al., 2007; Montemurro et al, 2006). Indeed, $\text{NAR}_{R\text{-}Com+Min}$ of maize is on average ten time higher than $NAR_{M\text{-}Com}$ it did not show negative value at first year of application only in combination with mineral fertiliser. Low N availability highlighted during the first year was probably due to the previous extended management of the soil with mineral fertilisers that needed a transitional period to re-activate the microbial community for efficient decomposition of the newly supplied organic matrix (Cong Tu et al., 2006; Iovieno et al., 2009; Sacco et al., 2015). However, even though microbial biomass increases and progressively specialised, it competes with N availability for crop (Evanylo et al., 2008) and N mineral application can mitigate the subsequent N stress.

4.2 Soil nitrogen quantity and forms

Since MSWC was characterized by a low C/N ratio and 80% of total N in soluble organic forms, STN increase was never measured over the experimental period neither in *M-Com* nor in *R-Com+Min.* Longer periods (Martínez-Blanco et al., 2013) and/or higher application rates (Alluvione et al., 2013; Evanylo et al., 2008; Hargreaves et al., 2008; Weber et al., 2014) are generally required to effectively raise STN values. The biodegradability of MSWC components is another important parameter that determines increase of STN (Chalhoub et al., 2013; Gale et al., 2006). In our study, the N content in the minimally biodegradable fraction of MSWC accounted for only 20% of total N (N content of NDF), corresponding to a total amount of 31 and 40 kg N $ha^{-1}year^{-1}$ applied every year in *R-Com+Min* and *M-Com,* respectively. Since STN content at the beginning of the experiment was 2485 kg N ha^{-1} for *R-Com+Min* (for 0-15 cm layer and with soil bulk density of 1.6 kg dm^{-3}), and 2537 kg N ha^{-1} for *M-Com* (in the same layer with the same bulk density), the contribution of N applied in stable form was negligible (12 and $15^{0}/_{00}$ of STN respectively).

However, with respect to treatments with mineral fertilisation, in which STN in 0-30cm layer decreased from 2010 to 2012 (-3% and -6% in *M-Min* and *R-Min* respectively), MSWC treatments did not decrease. Therefore, total N applied with MSWC was able to maintain initial STN level and was sufficient to counteract soil N losses, which occurred in mineral treatments, estimated as 101 kg ha^{-1}year^{-1} in the same layer of a commercial cropping system similar to *R-Min* and characterised by balanced mineral fertilisation (Sacco et al., 2015).

The maintenance of STN values with MSWC supply were consistent with N distribution in the different SON pools. The most stable organic fraction -that intimately bound to the mineral phase (MOM)- did not undergo any variation after MSWC application. However, a slight increase started in 0-30cm layer for MSWC applied alone, in which MOM *M-Com* results 8% higher than *M-Min*. Conversely, N in more labile organic fractions, both unprotected by stabilisation mechanisms (*f*POM) or occluded into soil aggregates (*o*POM) (Six et al., 2002) appeared significantly more concentrated in both MSWC fertilisation managements than when only mineral fertilisers were used. Since the high mineralisation of the soluble organic N forms in the top layer during the year following the first MSWC application has been widely proved (Chalhoub et al., 2013; Gale et al., 2006), after ploughing the N accumulation in 15-30 cm layer has not been detected. Instead, in 0-15cm layer, the average fPOM N in both MSWC treatments was 30% significantly greater than in mineral treatments. Both incorporation of such organic matrix and consequent microbial growth, as deduced by N immobilisation measured in the first year of MSWC application in *M-Com*, could have favoured aggregation processes and occlusion of supplied organic molecules in the newly formed aggregates, with a consequently appreciable increase of N *o*POM in the first layer (50% larger than mineral).

The offsetting of STN decrease performed by MSWC– instead evident in *Min* treatments- can be attributed to slow accumulation of *f*POM and *o*POM, main responsible for the gradual increase of maize yield or N content in crop tissues over time. The increase of more labile organic fractions in MSWC treatment (consequently the N availability especially for summer crops) were also confirmed by the higher PMN value in *R-Com+Min* than in *R-Min* and by the reduction throughout the three-year period of the ratio between yearly average of soil nitrate content in maize and soybean *R-Min* and *R-Com+Min* (from 1.28 to 1.04).

As already shown in the same pedoclimatic area for similar cereal cropping systems managed with other compost organic fertilisers (Sacco et al., 2015), a failure in synchronisation between N mineralisation activity and N uptake, more pronounced in winter wheat growth, can cause a slow potential fertiliser value of MSWC spread.

4.3 N_2O emissions and other potential losses

Compost was revealed an effective tool to mitigate N_2O emissions in this pedoclimatic zone (Alluvione et al., 2010). In our study, 65% (for wheat) or 75% (for maize) substitution of mineral N fertiliser with compost reduced emissions by 57 and 81%, respectively. When calculating the eco-efficiency of N_2O emissions, as the ratio between emitted N_2O and grain yield, 0.28 and 0.14 kg of $N\text{-}N_2O$ were emitted for each Mg of maize grain in *R-Min* and *R-Com+Min*, respectively, while 0.46 and 0.39 kg $N\text{-}N_2O$/Mg were emitted for each Mg of wheat grain for the two treatments. Despite yield decreased, compost was a valuable tool to combine agronomic productivity and environmental sustainability in terms of N_2O mitigation, especially in maize.

In compost-fertilised cropping system *R-Com+Min*, N potential losses are firstly indicated by the low maize NAR value. In the third year of these experiments (2012), 49% of MSWC N content (calculated as the complement to 100% of $NAR_{R\text{-}Com+Min}$) was, in fact, not used by crop. Given the very low ammonium content of MSWC and soil aerobic conditions, we assumed that volatilisation and complete denitrification were probably negligible. Therefore, this unused N amount can be considered in part transferred to PMN or *f*POM within 0-15 cm layer, lesser in the second soil layer and never in MOM fraction in any layers. Given the reduction of soil nitrate content observed between October and March in *R-Com+Min*, a considerable quota of the inefficient N could have been lost through leaching.

5 Conclusions

This study demonstrated that MSWC optimises its N fertiliser value when employed in spring-summer crops, maize especially, when it is repeatedly supplied, and integrated with mineral fertilisers. However, maize yields reduction can be very large, during the first two years of application in maize. In winter wheat yield reduction persists even in the third year.

Yields and N contents in crop tissues, NAR and NFRV trend identified N availability for plant as the main limiting factors of crop production. We advance three possible causes: i) soil N immobilisation at a high rate in particular during the first year of compost application, due to the development and progressive activity of specialised microbial biomass; ii) scarce synchronisation between available N release and N crop needs, more pronounced in winter wheat; iii) prevalent supply of soluble organic fractions that were rapidly mineralised during the year of application and did not merge in the most stable N organic fractions. This implies a mild cumulative residual N effect, which is measurable only with the combined application of mineral N fertilisers. Indeed, repeated compost applications increased the input of labile N fractions that enhanced grain and straw yields, N content in crop tissues, N apparent recovery and N fertiliser replacement value, but this occurred very slowly in MSWC employed alone and values never reached the levels of mineral treatments.

Therefore, MSWC fertilisation can show promising potential to provide available N for plants, if accompanied by an appropriate mineral integration to limit initial N immobilisation or deep deficiency during crop growth, or if applied to less N-demanding crops, like soybean.

Finally substituting a fraction of N supplied via mineral fertiliser with compost can mitigate N_2O emissions.

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Table 1: Mean value and coefficient of variation of chemical characteristics for all samples of composts collected prior of spreading. Dry matter is reported in g kg^{-1} of fresh matter. All other characteristics, except pH, are expressed in g kg^{-1} of DM.

Parameters	Average	CV (%)
Dry matter [a]	662	11
pH [b]	8	6
Total N [c]	22.3	12
Total P [d]	3.4	39
Total K [e]	10.1	39
Total C [f]	212.3	15
C/N	9.6	15
Neutral Detergent Fiber (NDF) [g]	311.6	11
Acid Detergent Fiber (ADF) [g]	219.5	14
Acid Detergent Lignin (ADL) [g]	149.5	11
Soluble C [h]	50.8	36
N-NDF [c]	4.5	63
Soluble N [i]	17.8	5
N-NH+4 [j]	0.4	78

Note: [a] Weight loss at 105 °C; [b] pH in water; [c] Kjeldahl method; [d] UV-VIS Spectrometry under continuous-flow conditions (Evolution II, Alliance) after mineralisation at 405 °C for 5h; [e] Atomic-absorption spectrometry after mineralisation at 405 °C for 5h; [f] Wet oxidation by dichromate; [g] From Van Soest et al. (1991); [h] computed as the difference between total C and C in NDF; [i] computed as the difference between Total N and N in NDF; [j] Steam distillation.

Table 2

Table 2: ROT trial. Seeding, and harvesting dates for all crops. Nitrogen, P_2O_5, and K_2O kg ha^{-1} year^{-1} supplied on average from 2010 to 2012 as compost (MSWC) and mineral fertilisers in the cropping systems.

Crops	Seeding date			Harvesting date			N-P_2O_5-K_2O			
	2010	2011	2012	2010	2011	2012	*R-Min*		*R-Com+min*	
							MSWC	Mineral	MSWC	Mineral
Maize	19/5	13/4	30/3	11/10	15/9	21/9	0-0-0	200-0-86	149-68-117	50-0-0
Winter wheat	31/10/09	11/11/10	20/10/11	9/7	28/6	6/7	0-0-0	140-0-150	93-60-69	50-0-93
Soybean	24/5	15/6	23/5	22/10	11/10	16/10	0-0-0	0-0-70	70-32-53	0-0-26

Table 3: MAIZE experiment. Grain and straw yields (Mg DM ha^{-1}) and N content (g N kg^{-1} DM). Means followed by different letters are statistically significant ($P<0.05$). Different letters for Interaction (Treatment * Year) denote different means of grain or straw ($P<0.05$) between treatments in each year.

	Part of plant	Treatment	2010		2011		2012		Mean		Effect	P(F)
Yield (Mg ha^{-1})	Grain	*M-Test 0*	6.9	B	4.0	b	4.7	c	5.2		Treatment	0.000
		M-Com	6.4	B	5.2	b	7.3	b	6.3		Year	0.001
		M-Min	15.0	A	13.3	a	12.6	a	13.6		Interaction	0.044
	Residue	*M-Test 0*	8.5		5.5		5.6		6.5	b	Treatment	0.000
		M-Com	7.8		6.7		7.4		7.3	b	Year	0.000
		M-Min	12.9		11.0		9.4		11.1	a	Interaction	n.s.
N (g kg^{-1})	Grain	*M-Test 0*	9.2		7.5		8.0		8.3	b	Treatment	0.000
		M-Com	9.2		6.7		7.5		7.8	b	Year	0.005
		M-Min	12.7		11.8		12.4		12.3	a	Interaction	n.s.
	Residue	*M-Test 0*	5.0		3.9		2.9		3.9	b	Treatment	0.000
		M-Com	5.0		4.2		3.2		4.1	b	Year	0.000
		M-Min	7.3		5.9		5.2		6.1	a	Interaction	n.s.

Note: Residue in maize refers to the sum of stalks, cobs, and bracts; block effect: P(F)=0.041 for yield straw; for all other cases it is always n.s.

Table 4: MAIZE experiment. Soil total nitrogen (STN), C/N ratio and Soil Organic Nitrogen (SON) fractions (*f*POM, *o*POM and MOM) at the onset (October 2009) and at the end of the experimental period (October 2012) for the first two parameters and only at the end for SON. Except C/N, all the values are expressed on dry soil. Different letters indicate different values between treatments on the line.

		M-Test 0			*M-Com*			*M-Min*			P(F) between (treat)	P(F) within (years)	P(F) Inter (treat*years)
	Depth cm	2009	2012		2009	2012		2009	2012				
STN (mg kg^{-1})	0-15	1095	994		1050	1069		1072	1008		ns	0,027	ns
	15-30	988	951		994	1056		991	993		ns	n.s.	ns
	30-60	721	583		696	559		709	650		ns	0,000	ns
C/N ratio	0-15	9,0	8,6		8,8	8,6		8,7	8,4		ns	ns	ns
	15-30	9,0	8,8		8,9	8,4		8,6	8,2		ns	0,002	ns
	30-60	8,3	8,0		8,2	8,0		7,8	7,9		ns	n.s.	ns
fPOM (mg N kg$^{-1)}$	0-15		24	b		50	a		21	b	0,001		
	15-30		21			24			17		ns		
	30-60		6			5			7		ns		
oPOM (mg N kg^{-1})	0-15		11	b		36	a		14	b	0,017		
	15-30		15			25			13		ns		
	30-60		4			22			5		ns		
MOM (mg N kg^{-1})	0-15		1018			974			934		ns		
	15-30		1025			1116			986		ns		
	30-60		712			736			672		ns		

Note: statistical analysis was conducted using repeated measure for STN and C/N; block effect STN: 0-15 cm P(F)= n.s., 15-30 cm P(F)= 0.006, 30-60 cm P(F)= n.s. C/N ratio 0-15 cm P(F)= 0.039, 15-30 cm P(F)= n.s., 30-60 cm P(F)= n.s.; *f*POM is free SON; *o*POM is the physically protected fraction; MOM is chemically protected fraction. For SON quality, one-way ANOVA was used to check only treatment in 2012.

Table 5: ROT trial. Grain yields and straw/residue biomass (Mg DM ha^{-1}) in the cropping systems. Means followed by different letters are statistically significant (P<0.05). Different letters for Interaction (System * Year) denote different means for grain or straw/residue (P<0.05) between cropping systems in each year.

Crop	Part of plant	System	2010	2011	2012	Mean	Effect	P(F)
Maize	Grain	*R-Min*	14.4a	13.6a	12.4a	13.5	System	0.000
		R-Com+Min	10.6b	11,5b	11.7a	11.3	Year	n.s.
							Interaction	0.022
	Residue	*R-Min*	13.2	9.7	10.0	11.0	System	n.s.
		R-Com+Min	11.3	9,5	10.1	10.3	Year	0.000
							Interaction	n.s.
Winter wheat	Grain	*R-Min*	5.4	4.2	6.7	5.4a	System	0.008
		R-Com+Min	4.4	3.0	4.8	4.1b	Year	0.004
							Interaction	n.s.
	Straw	*R-Min*	5.2	3.7	6.2	5.0a	System	0.007
		R-Com+Min	4.6	2.8	5.3	4.2b	Year	0.000
							Interaction	n.s.
Soybean	Grain	*R-Min*	3.5	3.7	3.9	3.7	System	n.s.
		R-Com+Min	3.7	3.1	3.8	3.5	Year	n.s.
							Interaction	n.s.
	Straw	*R-Min*	3.3	2.9	3.5	3.2	System	n.s.
		R-Com+Min	2.7	2.5	3.6	2.9	Year	0.003
							Interaction	n.s.

Note: Residue in maize refers to the sum of stalks, cobs and bracts; block effect: P(F)= 0.011 for grain soybean and P(F)= 0.005 for straw soybean; block effect is always n.s. for others crops in both parts of plants.

Table 6: ROT trial. Nitrogen content in grain and straw/residue of the different crops (g N kg^{-1} DM) in two cropping systems. Means followed by different letters are statistically significant ($P<0.05$). Different letters for Interaction (System * Year) denote different means of grain or straw/residue ($P<0.05$) between cropping systems in each year.

Crop	Part of plant	System	2010	2011	2012	Mean	Effect	P(F)
Maize	Grain	*R-Min*	12.5	12.1	13.1	12.6 a	System	0.000
		R-Com+Min	10.0	9.4	9.8	9.8 b	Year	n.s.
							Interaction	n.s.
	Residue	*R-Min*	8.0	6.7	5.6	6.8 a	System	0.000
		R-Com+Min	5.8	4.4	4.5	4.9 b	Year	0.000
							Interaction	n.s.
Winter wheat	Grain	*R-Min*	27.1 a	25.8 a	21.7 a	24.9	System	0.000
		R-Com+Min	17.0 b	19.3 b	16.4 b	17.6	Year	n.s.
							Interaction	0.027
	Straw	*R-Min*	6.2 a	4.5 a	3.8 a	4.8	System	0.000
		R-Com+Min	3.8 b	3.3 b	2.9 b	3.3	Year	0.003
							Interaction	0.043
Soybean	Grain	*R-Min*	72.7	69.6	62.1	68.2	System	n.s.
		R-Com+Min	67.8	68.6	63.0	66.5	Year	0.001
							Interaction	n.s.
	Straw	*R-Min*	11.1	7.8	5.0	8.0	System	n.s.
		R-Com+Min	10.1	6.5	6.7	7.8	Year	0.002
							Interaction	n.s.

Note: Residue in maize refers to the sum of stalks, cobs, and bracts; block effect is always n.s.

Table 7: ROT trial. Soil total nitrogen (STN), C/N ratio and Soil Organic Nitrogen (SON) fractions (*f*POM, *o*POM and MOM) in the two cropping systems measured at the onset (October 2009) and at the end of the experimental period (October 2012) for the first two parameters and only at the end for SON. Except C/N, all the values are expressed on dry soil. When effect was significant, different letters indicate different values between treatments on the line.

		R-Min		*R-Com+min*		P(F) between (System)	P(F) within (Years)	P(F) Interaction (System*years)
	Depth cm	2009	2012	2009	2012			
STN (mg kg^{-1})	0-15	982	906	1036	1081	0,020	n.s.	n.s.
	15-30	957	914	1035	1031	n.s.	n.s.	n.s.
	30-60	632	611	640	646	n.s.	n.s.	n.s.
C/N ratio	0-15	9.0	9.1	8.8	9.1	n.s.	n.s.	n.s.
	15-30	9.0	8.2	8.7	8.8	n.s.	n.s.	n.s.
	30-60	8.2	7.7	8.0	8.1	n.s.	n.s.	n.s.
fPOM (mg N kg$^{-1)}$	0-15		15b		24a	0,029		
	15-30		22		29	ns		
	30-60		4		8	ns		
oPOM (mg N kg^{-1})	0-15		5		9	ns		
	15-30		4		9	ns		
	30-60		3		2	ns		
MOM (mg N kg^{-1})	0-15		859		801	ns		
	15-30		905		843	ns		
	30-60		525		587	ns		

Note: statistical analysis was conducted using repeated measure for STN and C/N; block effect for STN: 0-15 cm P(F)= n.s., 15-30 cm P(F)= n.s., 30-60 cm P(F)= 0.015. Block effect is always n.s. for C/N ratio. *f*POM is free SON; *o*POM is the physically protected fraction; MOM is chemically protected fraction. For SON quality, one-way ANOVA was used to check only treatment in 2012.

Table 8: ROT trial. Potential Mineralisable Nitrogen (PMN) in the first soil layer (0-30 cm) at the end of the experimental period (October 2012) after winter wheat and maize.

Crop/System	PMN (mg N-NH_4 kg^{-1} soil)	PMN STN^{-1}(%)
Winter wheat	11.6b	1.30b
Maize	16.4a	1.74a
R-Min	11.4b	1.26b
R-Com+min	16.7a	1.79a
Effect P(F)		
System	0.000	0.000
Crop	0.001	0.001
Interaction	n.s.	n.s.

Figure 1

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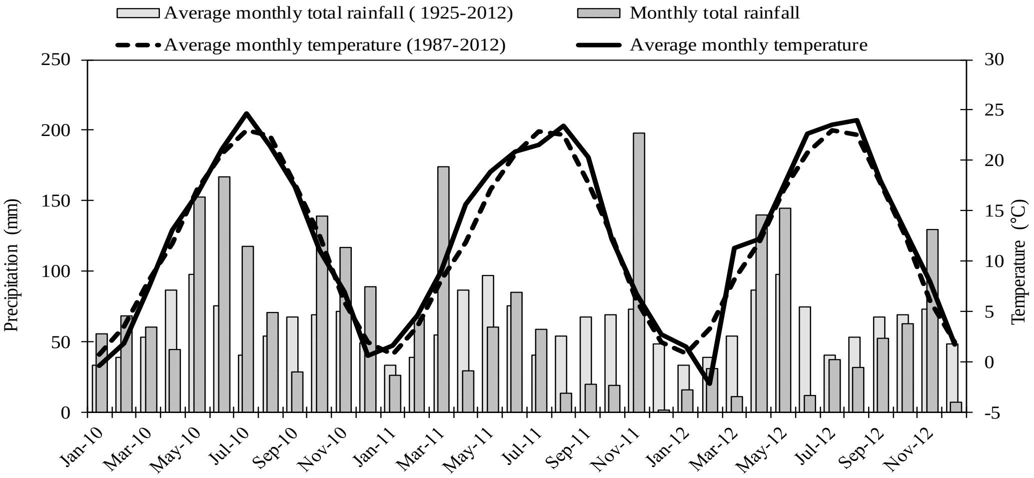


Figure 2

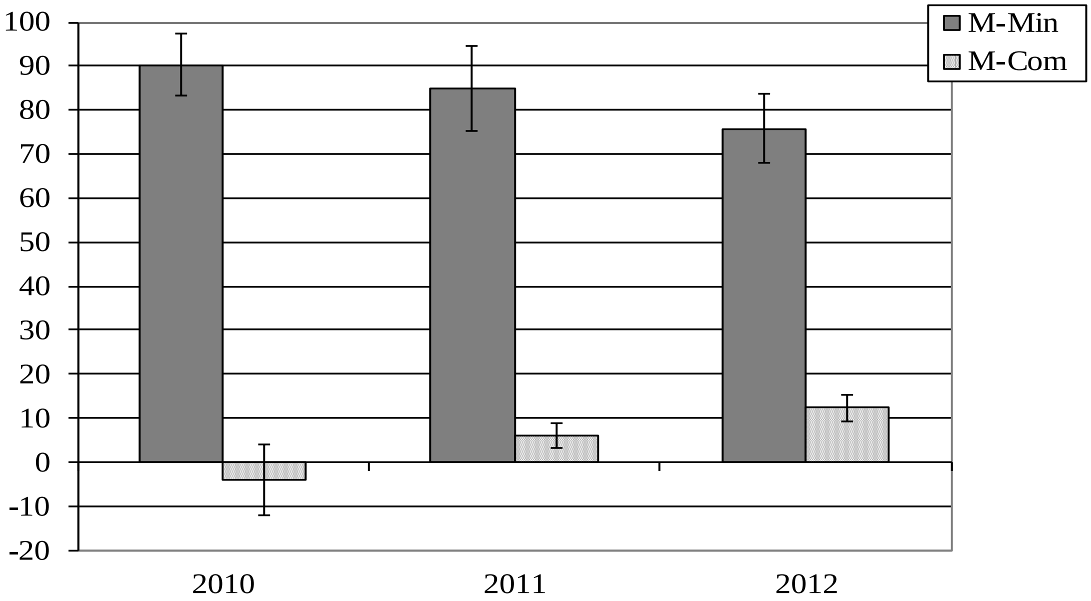
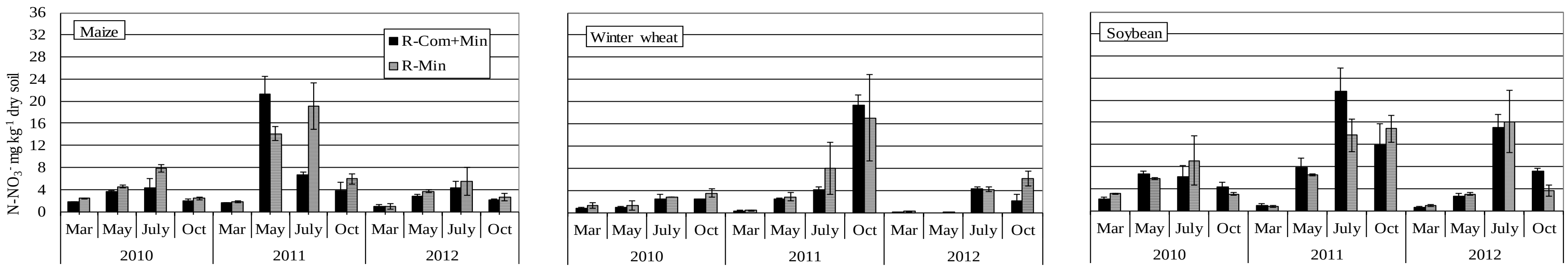


Figure 3


N-NO$_3^-$ mg kg^{-1} dry soil
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8
12
16
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24
28
32
36
Maize
■ R-Com+Min
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Winter wheat
Soybean
Mar
May
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Figure 4

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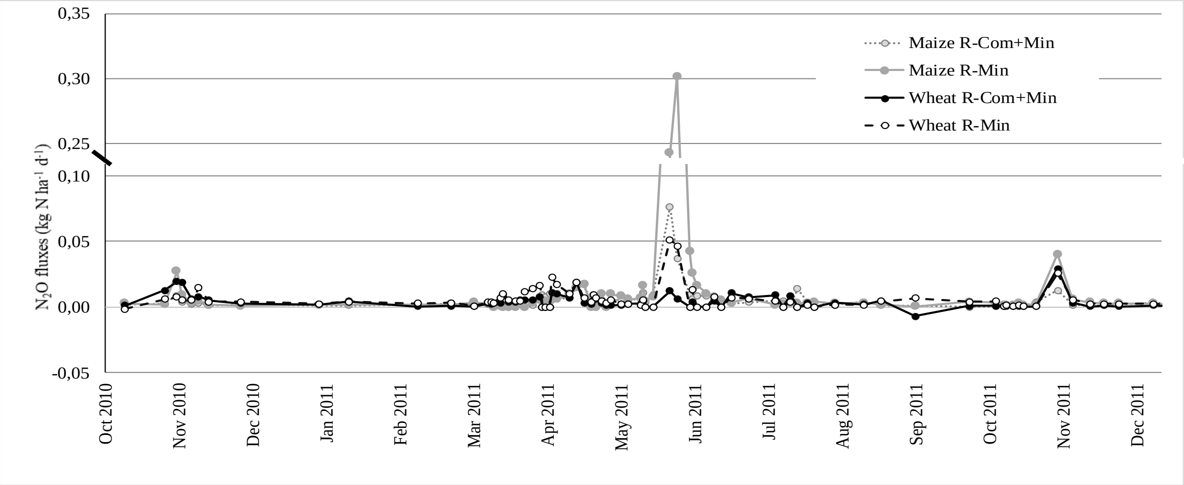

Figure 5

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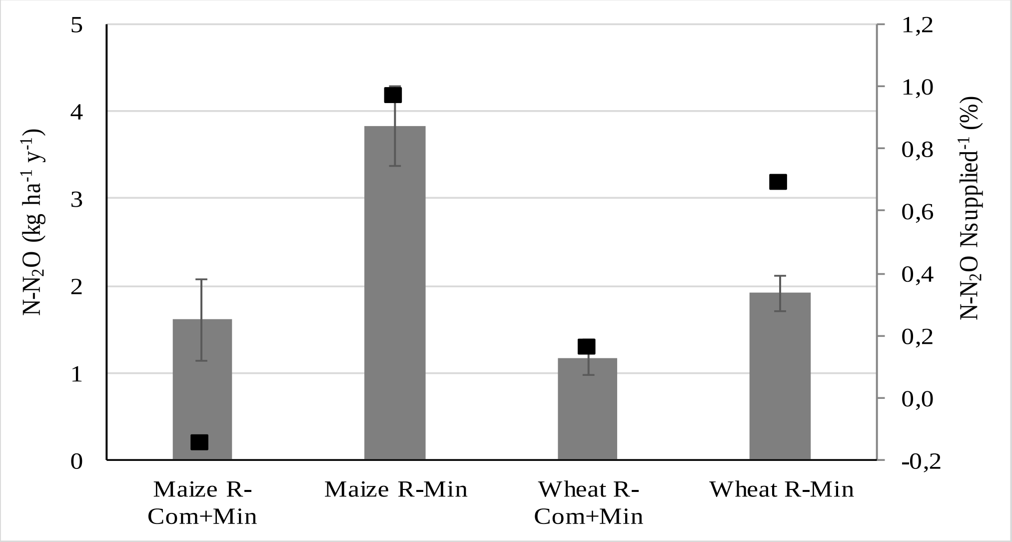
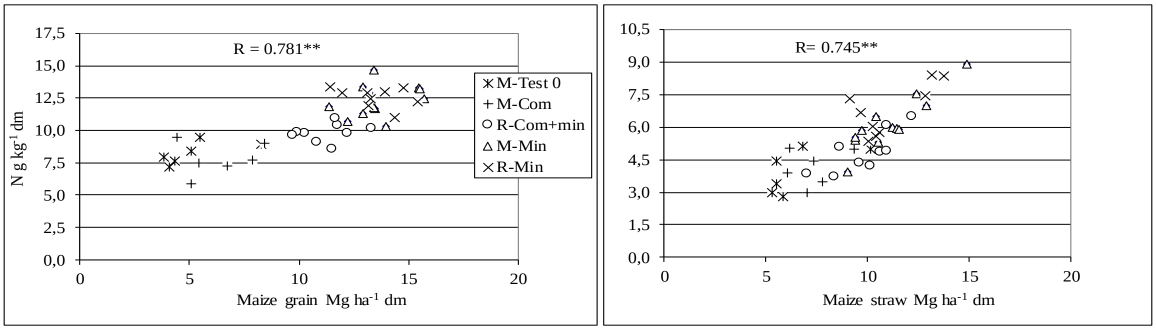


Figure 6


R = 0.781**
N g kg^{-1} dm
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15,0
12,5
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2,5
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0
5
10
15
20
Maize grain Mg ha^{-1} dm
✳ M-Test 0
+ M-Com
○ R-Com+min
△ M-Min
× R-Min
R= 0.745**
10,5
9,0
7,5
6,0
4,5
3,0
1,5
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5
10
15
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Maize straw Mg ha^{-1} dm

Captions

Figure 1: Climatic conditions. Average monthly temperature and precipitation during the 2010-2012 period and for longer climatic series.

Figure 2: MAIZE experiment NAR values. N apparent recovery (NAR) of *M-Com* ($NAR_{M\text{-}Com}$) and *M-Min* ($NAR_{M\text{-}Min}$) treatments compared in MAIZE experiment. Bars report standard error.

Figure 3: ROT trial soil nitrates. Soil $N\text{-}NO_3^-$ content (mg kg^{-1} dry soil) measured in the first layer (0-30 cm) during the three years and referred to all crops rotated in the cropping systems of the ROT trial. Bars refer standard error.

Figure 4: ROT trial nitrous oxide emission. Daily N_2O emissions from winter wheat and maize of both cropping systems (*R-Min* and *R-Com+Min*) compared in ROT trial.

Figure 5. ROT trial nitrous oxide accumulation. Yearly cumulative N_2O emissions (histogram) and emission factors (black square) from wheat and maize of both cropping systems (*R-Min* and *R-Com+Min*). For yearly cumulative N_2O emissions, maize crop: P(F) of cropping systems=0.009; block effect is n.s. Winter wheat crop: P(F) of cropping systems=0.031; block effect P(F) =0.034.

Figure 6: N content-yields correlations. Relationship between maize grain and straw N content with the respective grain and straw yields of all treatments (MAIZE experiment) and cropping systems (ROT trial) for the three-year period. Correlation was estimated with Pearson test considering the data of all three years.