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

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Keywords: Nitrogen apparent recovery, Nitrogen fertilizer replacement value, Nitrogen availability, Soil nitrates, Soil organic nitrogen fractionation, Nitrous oxide emissions.

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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 N₂O 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 N₂O emissions.

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

Sincerely,

Barbara Moretti

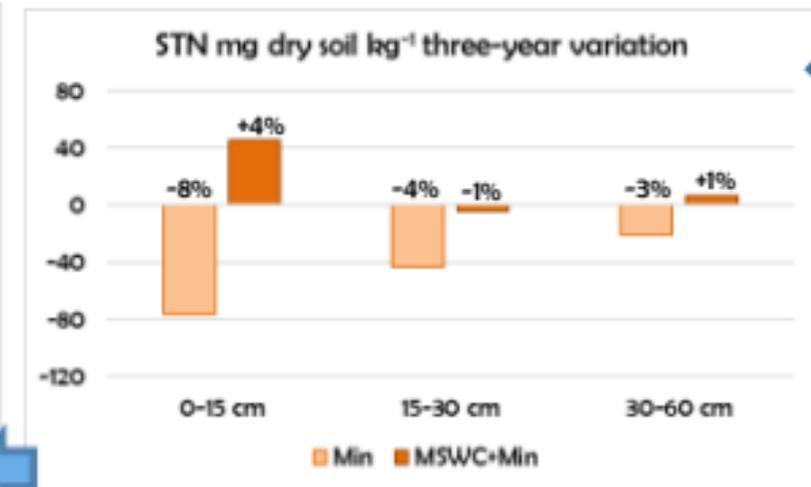
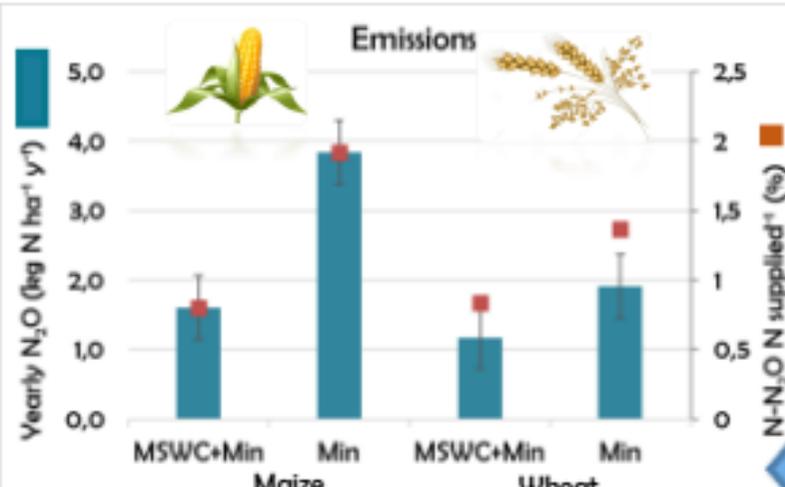
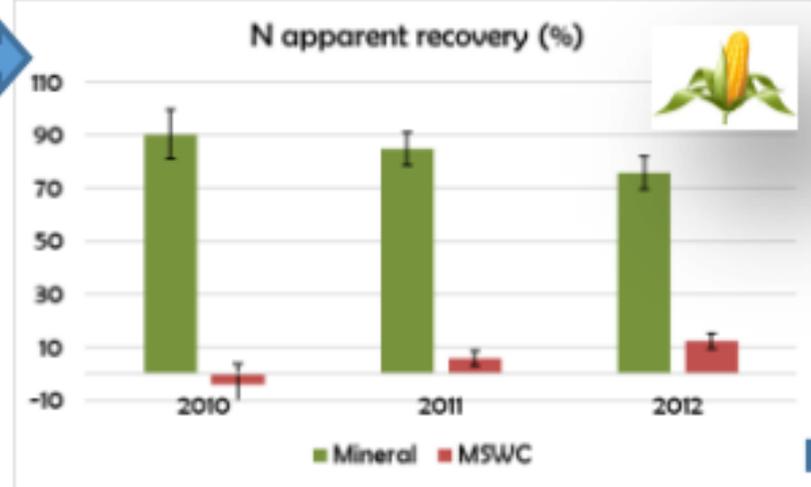
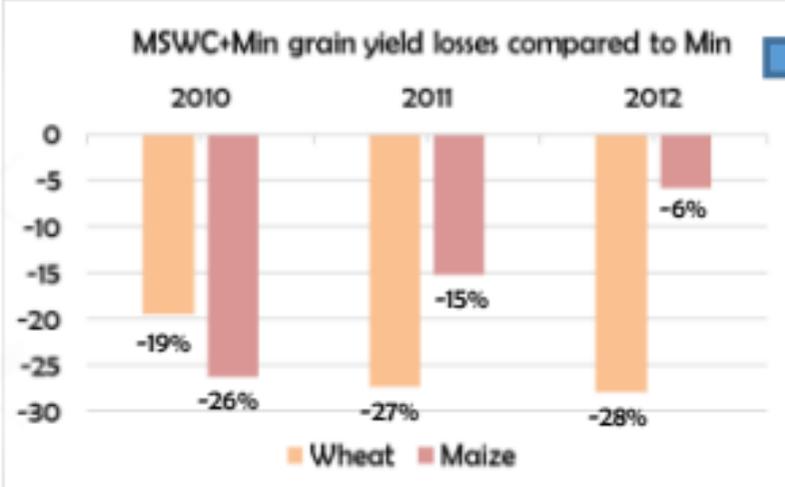
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Title:

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

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*Graphical Abstract



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 N₂O emissions compared to mineral fertilisation.

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

3

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24

25 **Abstract**

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

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48 nitrates, Soil organic nitrogen fractionation, Nitrous oxide emissions.

49

50 **1. Introduction**

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

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

66 The early reduction in N availability can be at least partially counteracted in the mid-term (two to
67 10 years) (Martínez-Blanco et al., 2013), benefitting from repeated applications, which contribute to
68 both select efficient microbial communities for degradation and mineralisation (García-Gil et al.,
69 2000) and build an organic stock of nutrients (Monaco et al., 2010). Some strategies can mitigate
70 this early yield loss. The combination of MSWC with N mineral fertilisers, for instance, certainly
71 improves the early N availability for crop, not only because of the presence of N in promptly
72 available forms, but also due to the stimulation that the added mineral N exerts on the soil microbial
73 community (Bhattacharyya et al., 2005; Busby et al., 2007; Montemurro et al., 2006).

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

81 Chemical-biochemical properties of compost itself influence significantly N mineralisation activity
82 (Busby et al., 2007; Chalhoub et al., 2013; Gilmour et al., 2003; Weber et al., 2014), and leads to
83 soil microbial community specialisation (Iovieno et al., 2009; Weber et al., 2014). Compost
84 characterised by a low C/N ratio generally increases soil N availability, even in the short term (Gale
85 et al., 2006; Gilmour and Skinner, 1999; Weber et al., 2014) due to a prevailing net N
86 mineralisation. In other cases, compost may cause an initial N immobilisation (Erhart et al., 2005;
87 Fiorentino et al., 2016; Hargreaves et al., 2008). This phenomenon usually occurs when the applied
88 compost has a high C/N ratio (>20), which reduces N availability in the short term (Chalhoub et al.,
89 2013; Gale et al, 2006) and increases the pool bound to soil organic matter (SOM), i.e. soil organic
90 N (SON). Repeated applications of MSWC may lead to enhanced N bioavailability later through
91 higher contributions of soluble inorganic N forms coming from microbial biomass and SON
92 mineralisation (Chalhoub et al., 2013; Diacono and Montemurro, 2010; Monaco et al., 2008).

93 Moreover, also the presence of highly-stabilised organic matter components can have lower N
94 mineralisable potentials if compared to low C/N and low-stabilised compounds (Gilmour et al.,
95 2003).

96 Furthermore, as with other organic fertilisers, the ability of compost to provide N to crop depends
97 also on the synchronisation of mineral N availability and N requirements by plants, otherwise
98 limiting N uptake and crop yield (Erhart et al., 2005, Evanylo et al., 2008, Montemurro et al., 2006).

99 Synchronisation between N availability and N uptake is pivotal not only for improving N use

100 efficiency, but also for minimising N losses. Sacco et al. (2015) showed similar potential N losses
101 in cropping systems managed with composted organic fertilisers and mineral fertilisers (76 and 101
102 kg ha⁻¹ year⁻¹, respectively) while Alluvione et al. (2010) found that MSWC application reduced
103 N₂O emissions (0.11% of applied N) compared to urea (3.4% of applied N).
104 Based on all these considerations, it is clear that MSWC application should be carefully managed to
105 optimise N uptake and use efficiency while limiting potential N losses. This work is aimed to
106 evaluate the effect of a three-year application of MSWC characterised by low C/N ratio and highly-
107 stabilised organic compounds in a continuous maize cropping system. The evaluation is addressed
108 to assess the MSWC N fertiliser value, i.e. its ability to sustain the N requirement of maize for grain
109 production and to enhance soil N availability. Then the results are used to interpret the application
110 of MSWC in combination with mineral fertilisers in a four-year rotation system typical of grain
111 commercial farms of the area. The combination of the two experiences was aimed to identify the
112 most appropriate MSWC application methods in terms of crop production and N efficiency while
113 limiting the environmental impact due to N losses from the agro-ecosystem.

114

115 **2. Materials and methods**

116 2.1 Site description

117 The present study is based on a continuous maize experiment (MAIZE experiment), and on a four-
118 year crop rotation trial (ROT trial). Both were located in the western Po River plain (NW Italy) in a
119 1.3 ha experimental platform (Lombriasco: 44° 50' 39" N, 7° 38' 15" E; 241 m a.s.l.) from 2010 to
120 2012.

121 According to Köppen-Geiger classification, the climate of the area is Cfa (Kottek et al., 2006) with
122 a mean annual temperature (from 2010 to 2012) of +12.5°C and an average minimum and
123 maximum monthly temperature of +9.1 and +19.1°C. The mean annual precipitation of the same

124 period was 846 mm with two main rainy periods during April-May and October-November. Figure
125 1 reports the main climatic parameters of the 2010-2012 period, compared to longer climatic series.
126 The soil was classified as loamy sand, coarse-loamy mixed non-acid mesic Typic Hapludalf
127 (USDA, 2010). The ploughed horizon (0-30 cm) contained 52 and 9% of sand and clay,
128 respectively, and was characterized by neutral pH (6.8). Soil organic carbon (SOC) was 8.9 g kg⁻¹
129 and C/N ratio was 9.0. Cation exchange capacity was 9.42 cmol₍₊₎ kg⁻¹, exchangeable K 67 mg kg⁻¹
130 and available P (P Olsen) was 67 mg kg⁻¹.

131

132 2.2 MAIZE experiment

133 2.2.1 Design and agronomic management

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

138 Three treatments, placed on plots of 4.2 m wide and 10.0 m long (42.0 m²), were set up in a
139 randomised complete block design with four replicates. The treatments varied in fertilisation
140 management as follows:

- 141 i) *M-Test 0*: Zero N, P, and K fertilisation, used as a control treatment;
- 142 ii) *M-Com*: MSWC supplied at average rate of 8.8 Mg of dry matter (DM) ha⁻¹ year⁻¹;
- 143 iii) *M-Min*: Total N, P₂O₅ and K₂O, supplied as mineral fertilisers (urea 46%, potassium
144 chloride 60%, and triple superphosphate 46%) and used for comparison.

145 The amount of nutrients supplied in the treatments was equal for both *M-Com* and *M-Min* (on
146 average 198, 98 and 126 kg ha⁻¹ year⁻¹ for N, P₂O₅ and K₂O respectively). The amount of MSWC
147 supplied in *M-Com* was set considering the N threshold indicated for maize grain production (200
148 kg ha⁻¹ year⁻¹) in Integrated Agriculture Techniques (IAT) of Regional Rural Development
149 Programmes (EC No 1698/2005) and the actual N content of compost, measured every year before

150 spreading. The amount of P_2O_5 and K_2O resulted from N/P_2O_5 and N/K_2O ratio specific for MSWC
151 applied. In *M-Min* P and K mineral fertilisers supply varied annually to achieve *M-Com*.
152 All treatments were mouldboard ploughed at 30 cm depth in autumn, to incorporate plant residues
153 of the previous year, and then disk harrowed twice in spring two days before seeding. All fertilisers,
154 either MSWC or minerals, were spread in spring before maize seeding and between the two disk
155 harrowing at the same time. Maize hybrid “SNK Famoso” (FAO rating 500; 127 days) was seeded
156 at a density of 7.1 plants/m². Seeding and harvesting dates were the same reported for maize of crop
157 rotation trial (Table 2). Drip irrigation supplied 40-60 mm of water two - four times yearly, as
158 needed. Only one post-emergence herbicide was applied at four leaves unfolded maize growth
159 stage.

160

161 2.2.2 Compost characteristics

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

174

175 2.2.3 Crop yields, N content and estimation of maize N availability indicators

176 For measuring yield and N concentration, maize grain and straw were manually collected at
177 physiological maturity from a total area of 12 m² (4 m x 2 rows with 0.75 m inter-row spacing x
178 two sampling areas) in each plot. Plant samples were oven-dried at 60°C for 72 hours and the dry
179 matter subsequently determined. Grain and straw total N was analysed by means of a CN elemental
180 analyser (Flash EA 1112, Thermoquest, MIPAF method, 2000, Italy).

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

183

$$184 \text{ NAR} = \{ [(Y*N)_{\text{Grain}} + (Y*N)_{\text{Residue}}]_{M\text{-}Min \text{ or } M\text{-}Com} - [(Y*N)_{\text{Grain}} + (Y*N)_{\text{Residue}}]_{M\text{-}Test 0} \} / Fc \text{ or } Fo$$

185 (1)

186

187 where Y is the maize grain and residue yield and N is the corresponding N tissue content (i.e. N
188 uptake of grain or residue) of *M-Min*, *M-Com*, or *M-Test 0*; Fc is the total N input (kg ha⁻¹) from
189 mineral fertilisers; Fo is the total N input (kg ha⁻¹) from MSWC.

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

192

193 2.2.4 Determination of soil N forms

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

198 Soil total N (STN) was measured as described for crop tissues above. Soil OM fractionation was
199 performed on the samples collected in October 2012 as described by Golchin et al. (1994) and
200 modified by Cerli et al. (2012). Briefly, 125 ml of Na-polytungstate (NaPT) solution (density 1.6 g
201 cm⁻³) was added to 25 g of soil. The suspension was gently hand shaken to ensure complete soil

202 wetting and to avoid aggregate disruption, then allowed to settle for 1 h. After centrifugation at
203 5600 rpm for 20 min, the light fraction (free particulate organic matter, *f*POM) was collected on a
204 0.7- μm glass microfibre filter, rinsed with deionized water, air dried, and finely ground. The
205 remaining soil was suspended again in fresh NaPT solution and treated ultrasonically (275 J ml⁻¹).
206 After centrifugation, the light fraction released from aggregate breakdown (occluded particulate
207 organic matter, *o*POM) was separated, washed, dried, and finely ground as described above.
208 Preliminary tests were performed to select the appropriate sonication energy to release the entire
209 pool of *o*POM, while avoiding contamination by minerals and the release of organic-mineral
210 complexes (Cerli et al., 2012). The remaining soil (mineral-associated organic matter, MOM) was
211 washed with deionized water until salt-free, then dried, and ground. Mass yields, as well as organic
212 C and N contents, were determined on all fractions.

213

214 2.3 ROT trial

215 2.3.1 Design and agronomic management

216 Analyses performed in the MAIZE experiment were extended to a trial with crop rotation system
217 (ROT, R-) similar to grain commercial farms of the Po river plain. ROT trial was organised as a
218 randomised complete block design and placed close to MAIZE experiment during the same period.
219 Therefore, climate and soil characteristics were the same of the first experimental trial. It compared
220 two cropping systems, characterised by four-year rotation of winter wheat (*Triticum aestivum* L.,
221 “Bologna” variety), maize for grain (*Zea mays* L., seeding the same hybrid of MAIZE experiment),
222 soybean (*Glycine max* L., “PR91M10” variety, maturity group 0+, reseeded in 2011 with
223 “Brillante” variety, maturity group 1-), and then maize for grain again. Each cropping system
224 included four plots of 12.6 m wide and 26.0 m long (328.0 m² each), managed with standard farm
225 machinery, in which the four crops were hosted simultaneously every year.

226 Both cropping systems were mouldboard ploughed to 30 cm in autumn and then disk harrowed
227 twice two days before seeding in autumn for winter wheat (236 kg ha⁻¹) and in spring for maize and

228 soybean (7.1 and 50 plants m⁻² respectively). In table 2 was reported seeding and harvesting date for
229 all crops.

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

- 232 i) *R-Min*: managed with mineral fertilisers as control;
233 ii) *R-Com+Min*: managed with a combination of MSWC (the same used in MAIZE
234 Experiment) and mineral N fertilisers.

235 Table 2 reports the amount of N, P₂O₅ and K₂O supplied as MSWC or mineral.

236 In *R-Min*, maize received 20% of urea (40 kg N ha⁻¹ year⁻¹) during seeding and the remaining 80%
237 (160 kg N ha⁻¹ year⁻¹) was split in top dressings between two tranches: at the eight leaf unfolded and
238 at the second detectable node. Winter wheat was top-dressed in spring with ammonium nitrate (27%
239 N) in two rates: at the beginning of tillering (60 kg N ha⁻¹ year⁻¹) and at the beginning of stem
240 elongation when the first node was at least one cm above tillering node (80 kg N ha⁻¹ year⁻¹). No N
241 fertilisation was supplied to soybean. No P fertiliser applications were needed for any crop as the
242 soil available P content was above the threshold (P_{Olsen}>20 mg kg⁻¹) set by IAT fertilisation
243 management. On the other hand, potassium chloride was distributed in a single application after the
244 first disk harrowing in autumn for winter wheat, and in spring for maize and soybean.

245 In *R-Com+Min*, MSWC was distributed and incorporated before the second disk harrowing in
246 autumn for winter wheat, and in spring for maize and soybean at a rate of 4.3, 6.6 and 3.0 Mg DM
247 ha⁻¹ year⁻¹, respectively. In addition to MSWC, 50 kg N ha⁻¹ of mineral fertiliser was applied at top-
248 dressing as ammonium nitrate for winter wheat at the beginning of tillering stage, and as urea for
249 maize at eight leaf unfolded stage. Consequently, N supplied via mineral fertilisers in wheat and
250 maize represented 37 and 25% of the total N applied on average for the three-year period,
251 respectively. No N mineral fertiliser was supplied to soybean. No P mineral integration was
252 required as MSWC contained sufficient P for all crops. Potassium chloride was supplied
253 simultaneously with MSWC, before the second disk harrowing, in autumn for winter wheat and in

254 spring for soybean (93 and 26 kg K₂O ha⁻¹ year⁻¹, respectively). No K mineral integration was
255 necessary for maize as the high amount of MSWC supplied and the balanced N/K₂O ratio was
256 adequate to maize uptake.

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

262

263 2.3.2 Crop yield, N content and estimation of maize N availability indicator

264 In the ROT trial, maize grain and straw were sampled from an area of 18 m² (4 m x 3 rows with
265 0.75 m inter-row spacing x two sampling areas) in each plot and collected as described for MAIZE
266 experiment. Grain and straw of winter wheat and soybean were mechanically collected by means of
267 a plot combine harvester from a total area of 18 m² (6 m length x 1.5 m width x two sampling areas)
268 in each plot.

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

273

274 2.3.3 Determination of soil N forms

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

278 Soil nitrate (N-NO₃) content was determined by collecting samples from the first layer (0-30cm) in
279 March, May, July, and October during the three cropping seasons (2010-2012) for each crop of both

280 cropping systems. Nitrate was extracted from soil with 1 M KCl for 1 h and determined by
281 colorimetry with a continuous flow analyser (Evolution II, Alliance Analytical Inc., Menlo Park,
282 CA).

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

288

289 2.3.4 Nitrous oxide emissions

290 A further analysis was carried out from October 2010 to January 2012 within ROT trial to estimate
291 N₂O emissions from the *R-Min* and *R-Com+Min* cropping systems, in the plots cropped with maize
292 and wheat. Emissions were measured applying a non-steady-state closed chamber technique
293 (Livingston and Hutchinson, 1995) with four replicates per cropping system. Stainless steel anchors
294 (75x36 cm for maize and 36x27 cm for wheat) were inserted 15 cm into the soil and remained there
295 throughout the experimental period, except for the period between tilling and seeding during which
296 they were removed for soil management. Wooden boards were adopted to access the anchors during
297 sampling to avoid soil compaction or crop disturbance. During each measurement event, a
298 rectangular stainless steel chamber (75x 36 x20 cm high for maize and 36x27x20 cm high for
299 wheat) was sealed to each anchor by means of a water-filled channel. Plants were never included in
300 the chamber headspace. Headspace gas samples internal headspace were collected by propylene
301 syringes at 0, 15, and 30 min after chamber closure. Thirty-milliliter air samples were then injected
302 into 12-mL evacuated vials, closed with butyl rubber septa (Exetainer1 vial from Labco Limited,
303 UK). Gas concentrations of the samples were determined by gas chromatography with a fully
304 automated gas chromatograph (Agilent 7890A), equipped with electron capture detector for N₂O
305 quantification. Fluxes were calculated from the linear or nonlinear (Hutchinson and Mosier, 1981)

306 increase in concentration (selected according to the emission pattern) in the chamber headspace
307 over time (Livingston and Hutchinson, 1995). Estimates of cumulative emissions for each plot were
308 based on linear interpolation across sampling days (Bertora et al., 2018).

309

310 2.4 Statistical analysis

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

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

319 Yearly cumulative N₂O emissions were analysed for wheat and maize of both ROT cropping systems
320 (*R-Min* and *R-Com+Min*) and ANOVA model included crop and system effects.

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

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

326

327 **3. Results**

328 3.1 MAIZE experiment

329 3.1.1 Crop yields and tissues N content

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

331 In terms of grain yield, *M-Min* was always the most productive. The *M-Com* treatment produced the
332 same as *M-Test 0* for the first two years. However, a significant interaction (treatment * year) was
333 present, as during the third year *M-Com* was the intermediate treatment and yielded 55% more than
334 *M-Test 0*. The *M-Min* treatment was the most productive also for plant residue. The same trend of
335 progressive increase of the initial difference between *M-Com* and *M-Test* was observed, but the two
336 treatments never differed significantly. Year effect revealed 2010 as the most productive for both
337 grain and straw. These high productions were attributed to the unusually high amount of rainfall
338 during the 2010 April-October growing period (581 mm, 267 mm, and 417 mm respectively in
339 2010, 2011, and 2012) if compared to the same period from 1925 to 2012 (Figure 1).
340 Grain and residue N concentrations did not reveal any interaction effect between treatment and
341 year. *M-Min* was always the highest and *M-Com* never differed from *M-Test 0*. Year 2010 showed
342 the highest concentrations.

343

344 3.1.2 Maize N apparent recovery

345 As shown in Figure 2, N apparent recovery of *M-Min* (NAR_{M-Min}) reduced progressively from 2010
346 to 2012 (90, 85 and 76% respectively). This was because of the progressive reduction of the
347 difference between *M-Min* and *M-Test 0* total N uptake (grain + straw) (179, 170, 151 kg ha⁻¹ for
348 2010, 2011 and 2012, respectively). Conversely, the NAR_{M-Com} values progressively increased from
349 2010 to 2012, due to a gradual increase of *M-Com* plant uptake.

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

352

353 3.1.3 Soil total N and SON fractions

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

355 Years induced a significant STN reduction from 2009 to 2012 in the first and in the third layers,
356 independently of fertilisation management. On the contrary, 2012 SON fractions in the first layer

357 revealed significant differences: N content in *f*POM and *o*POM were higher in *M-Com* than in *M-*
358 *Test 0* and *M-Min*, whereas the MOM fraction was unaffected by treatments. *M-Test 0* and *M-Min*
359 revealed the same values of SON fractions between different layers.

360

361 3.2 ROT trial

362 3.2.1 Crop yields and tissues N content

363 Maize grain yield revealed highly significant interaction effect of system by year (Table 5).

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

368 While winter wheat yields were significantly affected by both cropping system and year, for neither
369 grain nor straw yields interaction effect was significant. *R-Com+Min* grain and straw always
370 produced less than *R-Min*. On average, *R-Com+Min* yields were reduced 33 and 19% for grain and
371 straw with respect to *R-Min*. The higher rainfall occurred during the tillering stage in 2010 and 2011
372 (Figure 1) and the seeding delay in the same years (Table 3) induced the rank of yield by year
373 2012>2010>2011 for grain and straw. The two effects reduced shoot density in both systems as
374 shown by the mean values of 610 and 537 shoots/m² in 2010 and 2011, respectively, *versus* 899
375 shoots/m² in 2012 for the two cropping systems.

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

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

382 The N content of winter wheat tissues indicated a significant effect of the interaction system*year
383 both for grain and straw. Although *R-Com+Min* showed values consistently lower than *R-Min*, the
384 gap between the two cropping systems was reduced over the three-year period. Indeed, across 2010,
385 2011, and 2012, the N content in *R-Com+Min* was 59, 33, and 32% lower for grain and 63, 37, and
386 32% lower for straw than in *R-Min*, respectively.

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

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

392 Nitrogen apparent recovery (NAR) of maize cropped in *R-Com+Min*, measured considering *M-Test*
393 0 to estimate N natural availability for maize over the three-year period, showed an increase from
394 2010 to 2012 as follows: 34, 50 and 51%. Similar to NAR_{M-Min} , NAR_{R-Min} showed a progressive
395 decrease across the three-year: 91%, 88% and 82% respectively for 2010, 2011 and 2012.

396

397 3.2.2 Soil total N, SON fractions, and nitrates

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

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

406 Soil nitrate content measured for both cropping systems in the 0-30 cm layer (Figure 3) showed a
407 different behaviour among years and crops. In general, for all crops and cropping systems, values

408 were usually lower in March than in the previous October. With the exception of the peak measured
409 in July 2012 in soybean, values for the three crops were higher in 2011 than in the other two years,
410 corresponding to low rainfall from May to October (Figure 1). Comparing the three crops, the
411 greatest values were obtained with maize and soybean in late spring or summer (May-July), when
412 high temperatures promoted mineralisation activity. Furthermore, in soybean, higher values were
413 detected than in maize, as N fixation and low crop yield reduced crop uptake ($92 \text{ kg N ha}^{-1}\text{year}^{-1}$ in
414 soybean, after deduction of N derived from fixation, versus $202 \text{ kg N ha}^{-1} \text{ year}^{-1}$ in maize). On the
415 contrary, the absence of winter wheat crop uptake after the July harvest and the high mineralisation
416 of SOM and limited rainfall events caused the greatest values in autumn.

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

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

427

428 3.2. Nitrous oxide emissions

429 Nitrous oxide (N_2O) emission measurements covered both the wheat and maize cropping cycles and
430 intercropping periods in the ROT trial for a total of 15 months (Figure 4). The winter wheat firstly
431 peaked in November, a few weeks after seeding, earlier for *R-Com+Min* ($0.019 \text{ kg N ha}^{-1} \text{ d}^{-1}$) than
432 for *R-Min* ($0.015 \text{ kg N ha}^{-1} \text{ d}^{-1}$, one week later). An analogous intensification of fluxes was
433 observed in the subsequent autumn (November 2011) when peaks in the two cropping systems

434 occurred concurrently (0.029 and 0.026 kg N ha⁻¹ d⁻¹ for *R-Com+Min* and *R-Min*, respectively).
435 Shortly after vernalisation (at the beginning of tillering stage), the first top-dressing fertilisation
436 triggered emissions that produced a minor peak (0.010 and 0.009 kg N ha⁻¹ d⁻¹ for *R-Min* and *R-*
437 *Com+Min*, respectively). A more intense peak was subsequently observed only for *R-Min* (0.023 kg
438 N ha⁻¹ d⁻¹). The highest peak was reached at the beginning of stem elongation, just few days after
439 the last top-dressing N fertilisation, more intense in *R-Min* than in *Com+Min* (0.051 and 0.012 kg N
440 ha⁻¹ d⁻¹, respectively).

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

449 Yearly cumulative fluxes (from November 2010 to November 2011) were significantly affected by
450 both crop and system, with the highest values induced by *R-Min* on maize (3.84 kg N ha⁻¹ y⁻¹) and
451 lowest by *R-Com+Min* on wheat (1.18 kg N ha⁻¹ y⁻¹) (Figure 5). Emission factors expressed as kg
452 N-N₂O lost/kg N applied and calculated by subtracting the background N₂O emitted from an
453 unfertilised plot set apart for this use, were on maize 1.93 and 0.23 for *R-Min* and *R-Com+Min*, and
454 on wheat 0.96 and negative for *R-Min* and *R-Com+Min*, respectively.

455

456 **4. Discussion**

457 4.1 N availability for crop nutrition

458 Both grain and residue maize yields in *M-Com* (continuous maize experiment fertilised with
459 MSWC alone) were similar to treatment with no fertilisers supply. Since in *M-Com* the mean P
460 content of maize tissues resulted higher than in *M-Min* (3.1 and 2.0 g kg⁻¹ DM, respectively) and K
461 was similar (8.5 and 8.9 g kg⁻¹ DM, respectively), the N availability appeared to be the main
462 limiting factor of the maize yield reduction. To emphasise this, N content in *M-Com* grain and
463 residue was always lower than *M-Min*, and similar to *M-Test 0*. As a consequence, *M-Com* NAR
464 (5% average for three years) was always considerably lower than *M-Min* (84%) and even negative
465 in the first year of MSWC application.

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

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

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

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

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

496 As expected, MSWC behaved as any other composted organic fertilisers, like manure or
497 commercial organic fertilisers (poultry manure, manure, feather meal, and oleaginous residues
498 mixed) (Sacco et al., 2015). The most available N fraction (N-NH_4^+) in MSWC, indeed, accounted
499 only for about 2% of total N. The remaining N potentially plant absorbable pool probably derived
500 from the decomposition and mineralisation of the soluble organic fraction (80% of total N), which
501 decomposes faster than other organic components. Garnier et al. (2003) reported a constant
502 decomposition rate of 1.49 d^{-1} for the soluble organic fraction vs 0.10 d^{-1} for hemicelluloses, 0.15 d^{-1}
503 for cellulose, $2.22 \cdot 10^{-3} \text{ d}^{-1}$ for lignin, and $0.126 \cdot 10^{-3} \text{ d}^{-1}$ for humified organic matter. Therefore,
504 grain yield, together with N content in crop tissues, indicated that the application of compost
505 induces and take advantages of microbial activities of decomposition and mineralisation that are
506 more intense and useful during the maize growing period (late spring and summer) than during
507 winter wheat growing period (winter and spring). Thanks to a more intense N mineralisation and
508 longer synchronisation between N release and crop uptake, maize benefitted more from MSWC
509 fertilisation than winter wheat, especially after three years of application. In winter wheat, low

510 temperature at tillering growth stage (i.e. during January-February) led to a reduced available N
511 pool, inducing a low stem density and consequently a low grain yield. The N fertiliser value of
512 MSWC was thus unequal between crops, better performing on summer than on winter crops.

513 In this study, a progressive increase of MSWC for N availability in the three-year period could be
514 detected for three reasons. First, a continuous rise of grain yield during three-year period was
515 detected in *R-Com+Min* for maize reaching in the last year the productive level of *R-Min*. Second, a
516 gradual growth of maize NAR from the first to the third year both for *M-Com* and *R-Com+Min*
517 (+16 and +17%, respectively). Third, the gap of N content winter wheat tissues between
518 compost+mineral and mineral management, was reduced from -37 to -24% for grain and from -39
519 to -24% for straw from the first to the third year of the study. The N content of maize tissues did not
520 increase following the repeated addition of MSWC, neither in the experiment nor in rotation
521 cropping systems, because it is a high N-demanding crop (Zavattaro et al., 2012) and MSWC
522 applied in this study was unable to fully balance N maize requirements.

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

530 These results confirm that MSWC furnished better results for maize, if applied in combination with
531 mineral prompt fertilisers. Moreover, aside from the presence of microorganisms specialised to
532 organic material decomposition (due to previous management of ROT *Com+Min* plots), N mineral
533 supplement promotes mineralisation of the soluble organic fraction that enhances the availability of
534 this N source (Garnier et al., 2003), and consequently increase crop yield (Bhattacharyya et al.,
535 2005; Busby et al., 2007; Montemurro et al, 2006). Indeed, $NAR_{R-Com+Min}$ of maize is on average ten

536 time higher than NAR_{M-Com} it did not show negative value at first year of application only in
537 combination with mineral fertiliser. Low N availability highlighted during the first year was
538 probably due to the previous extended management of the soil with mineral fertilisers that needed a
539 transitional period to re-activate the microbial community for efficient decomposition of the newly
540 supplied organic matrix (Cong Tu et al., 2006; Iovieno et al., 2009; Sacco et al., 2015). However,
541 even though microbial biomass increases and progressively specialised, it competes with N
542 availability for crop (Evanylo et al., 2008) and N mineral application can mitigate the subsequent N
543 stress.

544

545 4.2 Soil nitrogen quantity and forms

546 Since MSWC was characterized by a low C/N ratio and 80% of total N in soluble organic forms,
547 STN increase was never measured over the experimental period neither in *M-Com* nor in *R-*
548 *Com+Min*. Longer periods (Martínez-Blanco et al., 2013) and/or higher application rates
549 (Alluvione et al., 2013; Evanylo et al., 2008; Hargreaves et al., 2008; Weber et al., 2014) are
550 generally required to effectively raise STN values. The biodegradability of MSWC components is
551 another important parameter that determines increase of STN (Chalhoub et al., 2013; Gale et al.,
552 2006). In our study, the N content in the minimally biodegradable fraction of MSWC accounted for
553 only 20% of total N (N content of NDF), corresponding to a total amount of 31 and 40 kg N ha⁻¹
554 year⁻¹ applied every year in *R-Com+Min* and *M-Com*, respectively. Since STN content at the
555 beginning of the experiment was 2485 kg N ha⁻¹ for *R-Com+Min* (for 0-15 cm layer and with soil
556 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
557 density), the contribution of N applied in stable form was negligible (12 and 15⁰/₁₀₀ of STN
558 respectively).

559 However, with respect to treatments with mineral fertilisation, in which STN in 0-30cm layer
560 decreased from 2010 to 2012 (-3% and -6% in *M-Min* and *R-Min* respectively), MSWC treatments
561 did not decrease. Therefore, total N applied with MSWC was able to maintain initial STN level and

562 was sufficient to counteract soil N losses, which occurred in mineral treatments, estimated as 101
563 kg ha⁻¹year⁻¹ in the same layer of a commercial cropping system similar to *R-Min* and characterised
564 by balanced mineral fertilisation (Sacco et al., 2015).

565 The maintenance of STN values with MSWC supply were consistent with N distribution in the
566 different SON pools. The most stable organic fraction -that intimately bound to the mineral phase
567 (MOM)- did not undergo any variation after MSWC application. However, a slight increase started
568 in 0-30cm layer for MSWC applied alone, in which MOM *M-Com* results 8% higher than *M-Min*.
569 Conversely, N in more labile organic fractions, both unprotected by stabilisation mechanisms
570 (*fPOM*) or occluded into soil aggregates (*oPOM*) (Six et al., 2002) appeared significantly more
571 concentrated in both MSWC fertilisation managements than when only mineral fertilisers were
572 used. Since the high mineralisation of the soluble organic N forms in the top layer during the year
573 following the first MSWC application has been widely proved (Chalhoub et al., 2013; Gale et al.,
574 2006), after ploughing the N accumulation in 15-30 cm layer has not been detected. Instead, in 0-
575 15cm layer, the average *fPOM* N in both MSWC treatments was 30% significantly greater than in
576 mineral treatments. Both incorporation of such organic matrix and consequent microbial growth, as
577 deduced by N immobilisation measured in the first year of MSWC application in *M-Com*, could
578 have favoured aggregation processes and occlusion of supplied organic molecules in the newly
579 formed aggregates, with a consequently appreciable increase of N *oPOM* in the first layer (50%
580 larger than mineral).

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

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

592

593 4.3 N₂O emissions and other potential losses

594 Compost was revealed an effective tool to mitigate N₂O emissions in this pedoclimatic zone
595 (Alluvione et al., 2010). In our study, 65% (for wheat) or 75% (for maize) substitution of mineral N
596 fertiliser with compost reduced emissions by 57 and 81%, respectively. When calculating the eco-
597 efficiency of N₂O emissions, as the ratio between emitted N₂O and grain yield, 0.28 and 0.14 kg of
598 N-N₂O were emitted for each Mg of maize grain in *R-Min* and *R-Com+Min*, respectively, while
599 0.46 and 0.39 kg N-N₂O/Mg were emitted for each Mg of wheat grain for the two treatments.
600 Despite yield decreased, compost was a valuable tool to combine agronomic productivity and
601 environmental sustainability in terms of N₂O mitigation, especially in maize.

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

611

612 **5 Conclusions**

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

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

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

632 Finally substituting a fraction of N supplied via mineral fertiliser with compost can mitigate N₂O
633 emissions.

634

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Table 1[Click here to download Table: Table 1.docx](#)

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[Click here to download Table: Table 2.docx](#)

Table 2: ROT trial. Seeding, and harvesting dates for all crops. Nitrogen, P₂O₅, and K₂O 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 ₂ O ₅ -K ₂ O			
	2010	2011	2012	2010	2011	2012	<i>R-Min</i>		<i>R-Com+min</i>	
							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[Click here to download Table: Table 3.docx](#)

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	<i>M-Test 0</i>	6.9 B	4.0 b	4.7 c	5.2	Treatment	0.000
		<i>M-Com</i>	6.4 B	5.2 b	7.3 b	6.3	Year	0.001
		<i>M-Min</i>	15.0 A	13.3 a	12.6 a	13.6	Interaction	0.044
	Residue	<i>M-Test 0</i>	8.5	5.5	5.6	6.5 b	Treatment	0.000
		<i>M-Com</i>	7.8	6.7	7.4	7.3 b	Year	0.000
		<i>M-Min</i>	12.9	11.0	9.4	11.1 a	Interaction	n.s.
N (g kg ⁻¹)	Grain	<i>M-Test 0</i>	9.2	7.5	8.0	8.3 b	Treatment	0.000
		<i>M-Com</i>	9.2	6.7	7.5	7.8 b	Year	0.005
		<i>M-Min</i>	12.7	11.8	12.4	12.3 a	Interaction	n.s.
	Residue	<i>M-Test 0</i>	5.0	3.9	2.9	3.9 b	Treatment	0.000
		<i>M-Com</i>	5.0	4.2	3.2	4.1 b	Year	0.000
		<i>M-Min</i>	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

[Click here to download Table: Table 4.docx](#)

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.

	Depth cm	<i>M-Test 0</i>		<i>M-Com</i>		<i>M-Min</i>		P(F) between (treat)	P(F) within (years)	P(F) Inter (treat*years)
		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
<i>f</i> POM (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	
<i>o</i> POM (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

[Click here to download Table: Table 5.docx](#)

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	<i>R-Min</i>	14.4a	13.6a	12.4a	13.5	System	0.000
		<i>R-Com+Min</i>	10.6b	11.5b	11.7a	11.3	Year	n.s.
							Interaction	0.022
	Residue	<i>R-Min</i>	13.2	9.7	10.0	11.0	System	n.s.
		<i>R-Com+Min</i>	11.3	9.5	10.1	10.3	Year	0.000
							Interaction	n.s.
Winter wheat	Grain	<i>R-Min</i>	5.4	4.2	6.7	5.4a	System	0.008
		<i>R-Com+Min</i>	4.4	3.0	4.8	4.1b	Year	0.004
							Interaction	n.s.
	Straw	<i>R-Min</i>	5.2	3.7	6.2	5.0a	System	0.007
		<i>R-Com+Min</i>	4.6	2.8	5.3	4.2b	Year	0.000
							Interaction	n.s.
Soybean	Grain	<i>R-Min</i>	3.5	3.7	3.9	3.7	System	n.s.
		<i>R-Com+Min</i>	3.7	3.1	3.8	3.5	Year	n.s.
							Interaction	n.s.
	Straw	<i>R-Min</i>	3.3	2.9	3.5	3.2	System	n.s.
		<i>R-Com+Min</i>	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[Click here to download Table: Table 6.docx](#)

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	<i>R-Min</i>	12.5	12.1	13.1	12.6 a	System	0.000
		<i>R-Com+Min</i>	10.0	9.4	9.8	9.8 b	Year	n.s.
							Interaction	n.s.
	Residue	<i>R-Min</i>	8.0	6.7	5.6	6.8 a	System	0.000
		<i>R-Com+Min</i>	5.8	4.4	4.5	4.9 b	Year	0.000
							Interaction	n.s.
Winter wheat	Grain	<i>R-Min</i>	27.1 a	25.8 a	21.7 a	24.9	System	0.000
		<i>R-Com+Min</i>	17.0 b	19.3 b	16.4 b	17.6	Year	n.s.
							Interaction	0.027
	Straw	<i>R-Min</i>	6.2 a	4.5 a	3.8 a	4.8	System	0.000
		<i>R-Com+Min</i>	3.8 b	3.3 b	2.9 b	3.3	Year	0.003
							Interaction	0.043
Soybean	Grain	<i>R-Min</i>	72.7	69.6	62.1	68.2	System	n.s.
		<i>R-Com+Min</i>	67.8	68.6	63.0	66.5	Year	0.001
							Interaction	n.s.
	Straw	<i>R-Min</i>	11.1	7.8	5.0	8.0	System	n.s.
		<i>R-Com+Min</i>	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

[Click here to download Table: Table 7.docx](#)

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.

	Depth cm	<i>R-Min</i>		<i>R-Com+min</i>		P(F) between (System)	P(F) within (Years)	P(F) Interaction (System*years)
		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.
<i>f</i> POM (mg N kg ⁻¹)	0-15		15b		24a	0,029		
	15-30		22		29	ns		
	30-60		4		8	ns		
<i>o</i> POM (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[Click here to download Table: Table 8.docx](#)

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
<i>R-Min</i>	11.4b	1.26b
<i>R-Com+min</i>	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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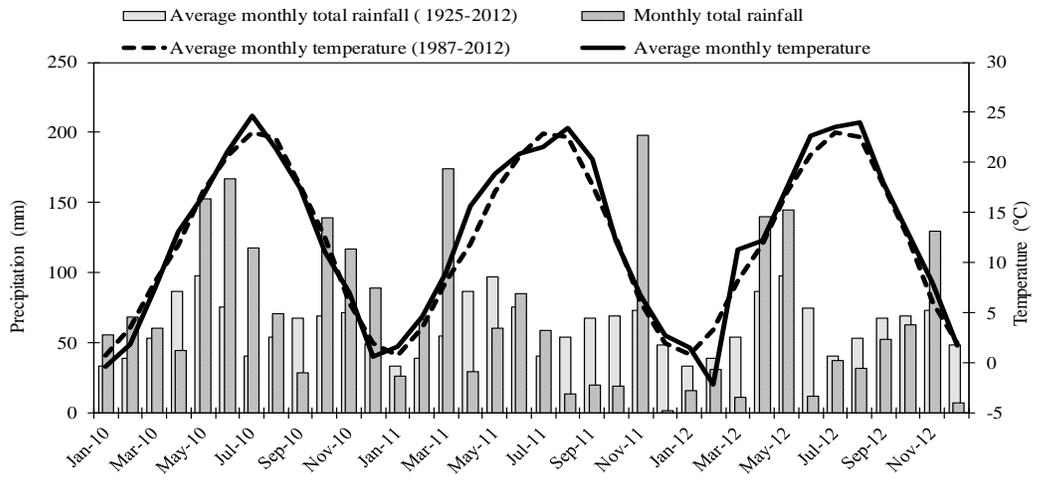


Figure 2
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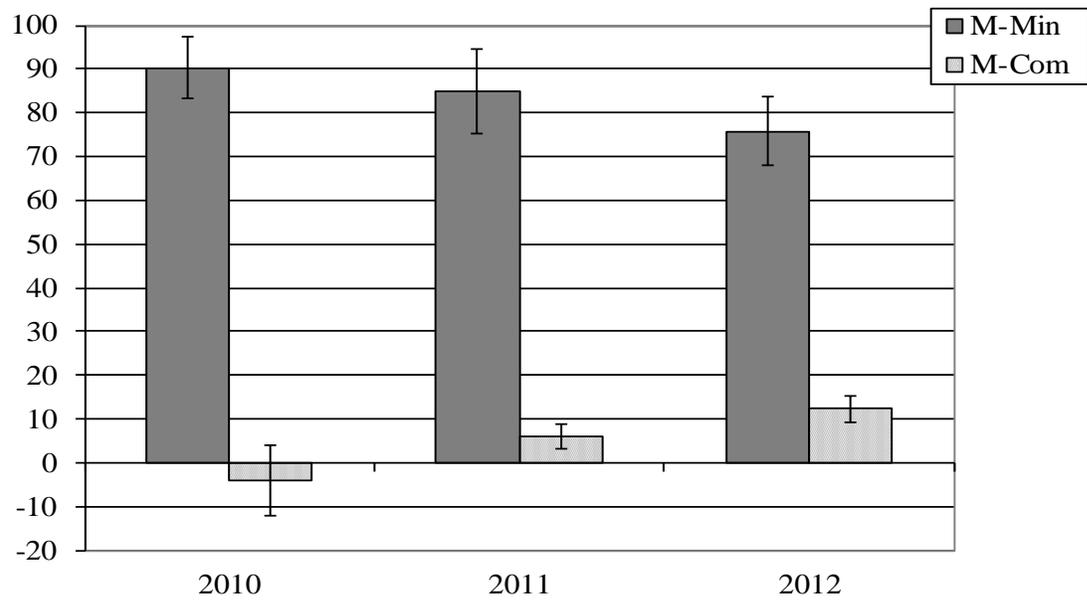


Figure 3
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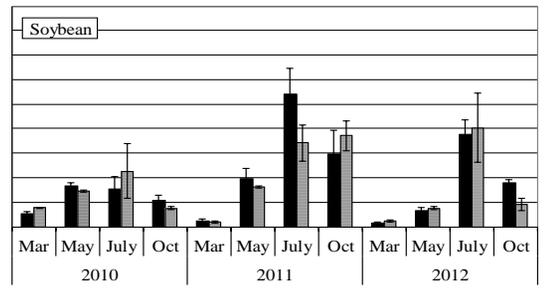
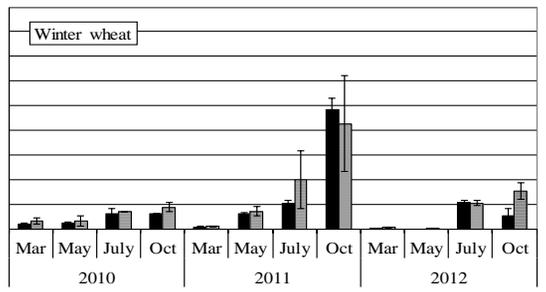
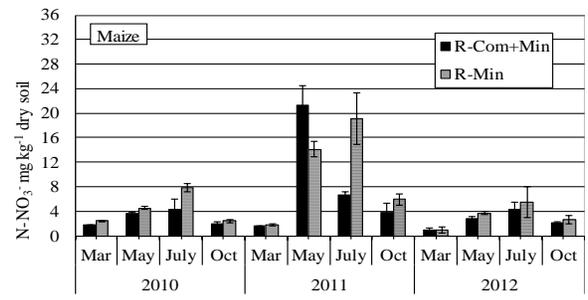


Figure 4
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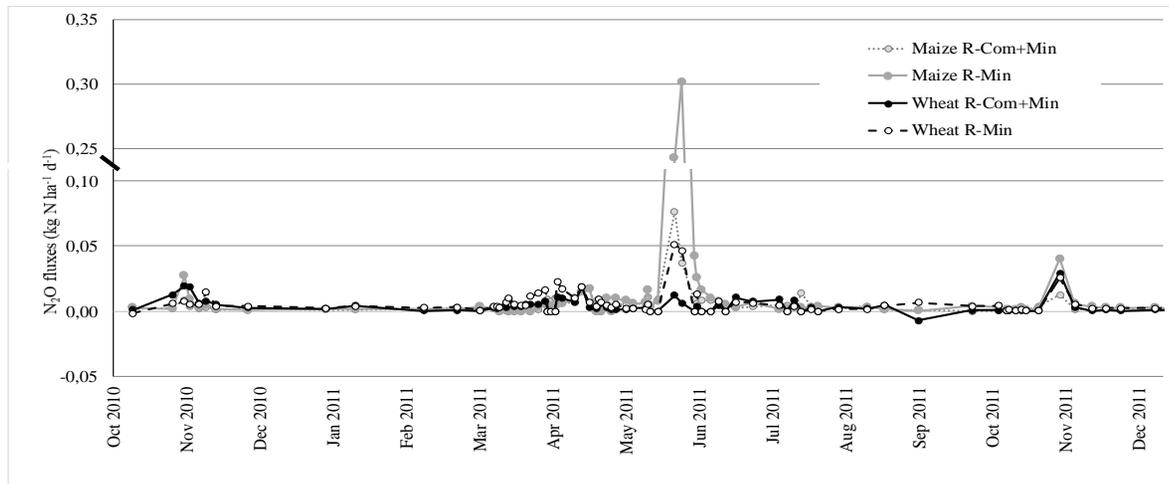


Figure 5

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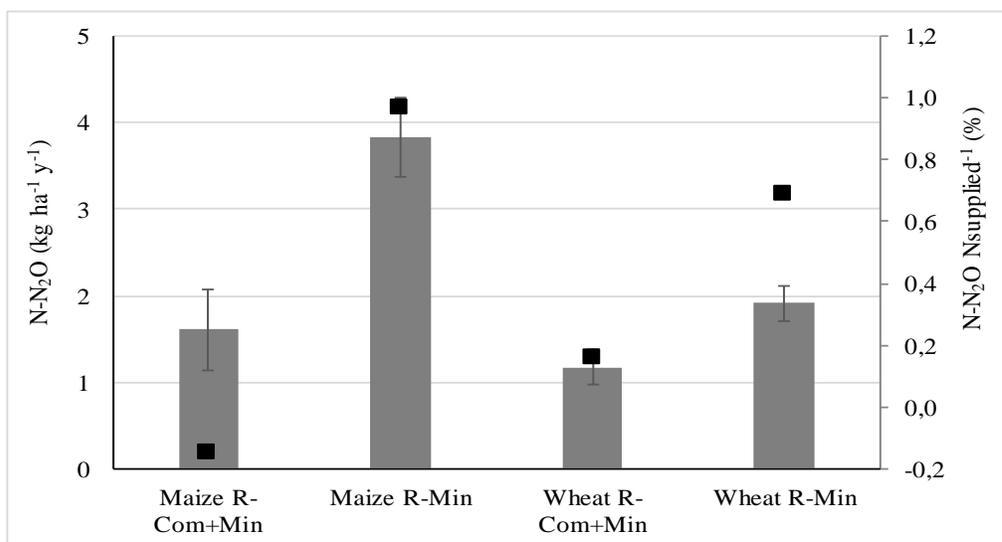
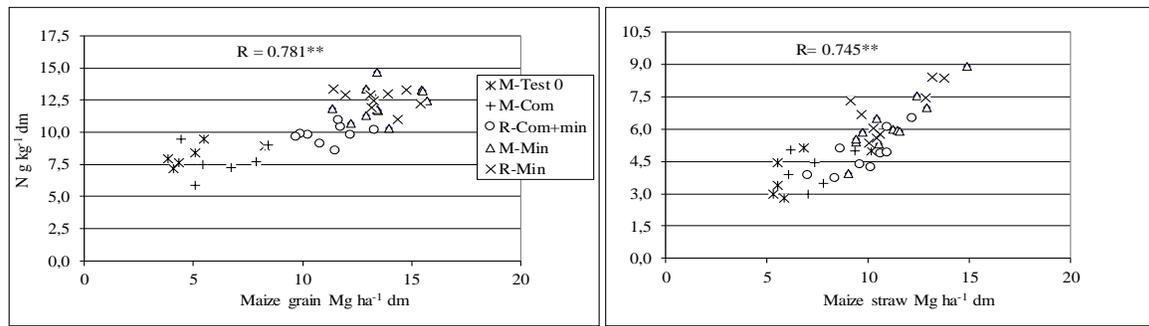


Figure 6

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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-Com}) and *M-Min* (NAR_{M-Min}) treatments compared in MAIZE experiment. Bars report standard error.

Figure 3: ROT trial soil nitrates. Soil $N-NO_3^-$ 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.