Renewable Agriculture and Food Systems

Green manure and phosphorus fertilization affect weed community composition and crop/weed competition in organic maize --Manuscript Draft--

Manuscript Number:	RAFS-D-18-00135R2
Full Title:	Green manure and phosphorus fertilization affect weed community composition and crop/weed competition in organic maize
Article Type:	Research Paper
Keywords:	hairy vetch; compost; soil amendment; Integrated Weed Management; weed diversity
Corresponding Author:	Stefano Carlesi, Ph.D. Scuola Superiore Sant'Anna Pisa, Toscana ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Scuola Superiore Sant'Anna
Corresponding Author's Secondary Institution:	
First Author:	Stefano Carlesi, Ph.D.
First Author Secondary Information:	
Order of Authors:	Stefano Carlesi, Ph.D.
	Federica Bigongiali
	Daniele Antichi
	Corrado Ciaccia
	Fabio Tittarelli
	Stefano Canali
	Paolo Bàrberi
Order of Authors Secondary Information:	
Abstract:	<p>Green manure and compost enriched in phosphorus can promote the sustainability of cropping systems by increasing soil fertility over the long term. They can also be used to manage crop/weed interactions, a key element in guaranteeing a proper level of satisfactory crop yields. We studied how green manuring with hairy vetch (<i>Vicia villosa</i> Roth.) and the application of different phosphorous-enriched compost affect weed/maize (<i>Zea mays</i> L.) interactions in an organic stockless Mediterranean agroecosystem for two consecutive dry years. Green manure stimulated the expression of maize traits related to a higher competitive ability against weeds, such as early growth, height and leaf area index, while the effect of compost was less clear. Regarding crop/weed competition, both green manuring and a phosphorus enriched compost application gave a significant advantage to maize. Neither green manure nor the compost increased total weed density and biomass compared to the control. Green manuring, significantly affected the weed community composition. The relative density of ruderal and competitive-ruderal species (according to Grime's classification) was higher in plots where the green manure was applied. The use of green manure, together with novel composting techniques, significantly affected crop/weed competitive interactions, favouring maize, but also creating favourable conditions for unwanted weed species such as competitive-ruderals. Increasing nitrogen availability the early growth stages of maize through green manuring can increase crop competitive ability. However but this may not suffice to preserve the system from future weed problems, should potentially detrimental species be selected. Dedicated strategies for the control of emerging weed species may thus be needed.</p>

Green manure and phosphorus fertilization affect weed community composition and crop/weed competition in organic maize

Authors:

Stefano Carlesi¹, Federica Bigongiali¹, Daniele Antichi², Corrado Ciaccia³, Fabio Tittarelli³,
Stefano Canali³, Paolo Bàrberi¹

¹Institute of Life Sciences, Scuola Superiore Sant'Anna - Piazza Martiri della Libertà 33 -56127 Pisa, Italy.

²Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

³Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria. Centro di ricerca Agricoltura e Ambiente (CREA - AA), Via della Navicella, 2 - 00184, Roma (RM), Italy.

Corresponding author: Stefano Carlesi:

email: s.carlesi@santannapisa.it

tel +39 050 88 3569

fax + 39 050 88 3526

1 Green manure and phosphorus fertilization affect weed community composition 2 and crop/weed competition in organic maize

3

4 **Keywords:** hairy vetch, compost, soil amendment, Integrated Weed Management, weed
5 diversity

6

7 **Abstract**

8 Green manure and compost enriched in phosphorus can promote the sustainability of
9 cropping systems by increasing soil fertility over the long term. They can also be used to
10 manage crop/weed interactions, a key element in guaranteeing an appropriate level of
11 satisfactory crop yields. We studied how green manuring with hairy vetch (*Vicia villosa*
12 Roth.) and the application of different types of phosphorous-enriched compost affect
13 weed/maize (*Zea mays* L.) interactions in an organic stockless Mediterranean
14 agroecosystem for two consecutive dry years. Green manure stimulated the expression
15 of maize traits related to a higher competitive ability against weeds, such as early
16 growth, height and leaf area index, while the effect of compost was less clear. Regarding
17 crop/weed competition, both green manuring and a phosphorus enriched compost
18 application gave a significant advantage to maize. Neither green manure nor compost
19 increased total weed density and biomass compared to the control. Green manuring
20 significantly affected the weed community composition. The relative density of ruderal
21 and competitive-ruderal species (according to Grime's classification) was higher in plots
22 where the green manure was applied. The use of green manure, together with novel
23 composting techniques, significantly affected crop/weed competitive interactions,
24 favouring maize, but also creating favourable conditions for unwanted weed species

1 such as competitive-ruderals. Increasing nitrogen availability in the early growth stages
2 of maize through green manuring can increase crop competitive ability. However, this
3 may not suffice to preserve the system from future weed problems, should potentially
4 detrimental species be selected. Dedicated strategies for the control of emerging weed
5 species may thus be needed.

6

7 **Word count :**

8 **Abstract: 255**

9 **Main text 5263**

10 **References: 1707**

11

12 **Introduction**

13 The presence of weeds throughout the cropping cycle of maize (*Zea mays* L.) can reduce
14 the crop yield in many different ways, for example by competing for water, mineral
15 nutrients and light (Rajcan and Swanton, 2001). Competition is important especially at
16 early crop development stages, but may also have consequences at later stages. For
17 instance, Tollenaar *et al.* (1994) reported reduced ear leaf chlorophyll concentration at
18 the silking stage of maize grown under early high weed pressure compared to weed-free
19 maize. Thomas and Allison (1975) also observed lower maize root development with
20 weed interference compared to weed-free conditions.

21 In organic farming, weed control in maize can be extremely challenging and relies on an
22 Integrated Weed Management (IWM) strategy through the application of both direct
23 (e.g. mechanical tools or flaming) and indirect methods (Bàrberi, 2002). Indirect
24 methods include all crop practices that prevent high weed occurrence while

1 simultaneously stimulating crop growth and competitive ability. In this context, nitrogen
2 (N) and phosphorus (P) fertilization may play a crucial role (Rajcan and Swanton, 2001;
3 Grant *et al.*, 2001). In fact, crop management recommendations rarely consider that
4 added nutrients might enhance weed growth as well as crop growth, resulting in
5 crop/weed competitive interactions which are potentially detrimental for the crop yield
6 (Blackshaw *et al.*, 2004; Blackshaw and Brandt, 2008). Information on how each
7 nutrient type affects crop/weed competition might help to improve IWM systems. N and
8 P availability may affect the maize capacity to compete with weeds, by favouring the
9 expression of plant traits that are more related to a higher competitive ability, e.g. leaf
10 area and plant height. Mohammadi (2007) showed that leaf area is one of the most
11 reliable parameters to estimate maize competitive ability against weeds, while Zystro *et*
12 *al.* (2012) found that plant height was the best predictor for estimating the weed
13 suppressive ability of sweet maize varieties.

14 In organic stockless cropping systems, where it is not possible to amend the soil with
15 raw animal effluents or farmyard manure, N and P could be supplied by soil
16 incorporation of green manures, composts, and natural fertilizers such as rock
17 phosphates.

18 Weed suppression and nutrient (especially N) release are two of the many benefits
19 potentially provided by green manures. Legume green manures, such as hairy vetch
20 (*Vicia villosa* Roth.), supply N to the soil through symbiotic N₂-fixation and increase soil
21 P availability by releasing acid root exudates (Karasawa and Takahashi, 2015) and/or
22 hosting symbioses with arbuscular mycorrhizal fungi (AMF) (Njeru *et al.*, 2014). Hairy
23 vetch is one of the most efficient N-fixing crops (e.g. Hartwig and Ammon, 2002), which
24 accumulates a large amount of N during its growing cycle (Anugroho *et al.*, 2009).About

1 50% of this N is readily available for the subsequent cash crop after soil incorporation
2 (green manuring) (Brandsæter *et al.*, 2008), but also for the field weed community
3 (Bittencourt *et al.*, 2013).

4 While the effect of fertilization on weed composition has been investigated in several
5 studies (Tang, *et al.*, 2014), little information is available on the effect of hairy vetch
6 green manure on weed community composition in the subsequent cash crop. Legume
7 green manures can change weed communities thanks to the release of allelopathic
8 compounds (Bittencourt *et al.*, 2013) and by reducing the amount of resources available
9 to weeds during the legume green manure growing season (Reddy and Koger, 2004).

10 Weed seedling emergence and biomass after soil incorporation of a legume cover crop
11 can be affected by the availability of residual N mineralized from the cover crop biomass,
12 although this effect varies according to the weed species (Sweeney *et al.*, 2008).

13 Soil P levels may affect weed as well as crop growth, thus crop/weed competitive
14 interactions may be influenced by P management (Blackshaw and Brandt, 2009).

15 Nitrogen and phosphorus are absorbed throughout a plant's growing cycle, but the
16 initial stages of development are the most critical. The availability of N is crucial in
17 sustaining maize yield and should be provided at all growing stages, including the grain
18 filling periods, especially for late season genotypes grown under wet conditions (Rajcan
19 and Swanton, 2001). In dry climates, N availability at early stages (from emergence to 5-
20 6 leave stage) is key in supporting maize growth, as it can counteract the negative effects
21 of drought (de Oliveira *et al.*, 2018). For P, availability in the soil between the maize
22 sowing and six-leaf stage is fundamental for a good establishment of the crop, as P
23 deficiency can slow down the appearance of new leaves (Grant *et al.*, 2001).

1 Weed species vary considerably in their ability as P scavengers (Blackshaw *et al.*, 2004).
2 Weed biomass responds positively to increasing amounts of soil P (Hoveland *et al.*,
3 1976), but the magnitude of responses varies markedly across species (Lehoczky *et al.*,
4 2015; Owla *et al.*, 2015). Blackshaw *et al.* (2004) found that P fertilization can have a
5 large impact on weed growth and that soil P levels affect crop/weed competitive
6 interactions. In particular, weeds seem more sensitive to low P and K levels than crop
7 species (Hoveland *et al.*, 1976). Despite studies on the potential influence of P on weed
8 populations and crop/weed competitive relationships, there is a lack of information on
9 how weed species respond to organic sources of P (e.g. composts naturally rich in P or
10 enriched with high P-content materials) and on the combined use of such composts with
11 green manure.

12 We conducted a field experiment on a long-term stockless rainfed arable crop rotation.
13 The aim was to investigate the effect of the combined use of a hairy vetch cover crop as
14 winter green manure with different types of composts on weed suppression and weed
15 community composition in a subsequent maize crop. We wanted to verify whether the
16 combined use of hairy vetch and P-enriched compost: (i) enhances the competitive
17 ability of organic maize by supplying the crop with sufficient levels of N and P and by
18 sustaining crop growth especially at early stages; (ii) reduces weed competitiveness by
19 reducing weed emergence and (iii) affects weed species composition.

20

21 **Materials and methods**

22 To test our three hypotheses, a field trial was established at the Centre for Agri-
23 Environmental Research "Enrico Avanzi" (CiRAA) of the University of Pisa (Italy) in
24 2010/2011 and 2011/2012. The area has a coastal Mediterranean climate with a mean

1 annual precipitation of 844 mm and a mean annual temperature of 15°C. Precipitation is
2 mainly in autumn and early spring, whilst summers are usually dry. In the two
3 experimental years precipitation was exceptionally low (Annex 1), especially in 2011,
4 with negative effects on biomass production of cash crops and weeds.

5 The soil is a loamy typic Xeropsamment (Mazzoncini *et al.*, 2010). The trial was arranged
6 in two fields as part of a long-term experiment named MASCOT (Bàrberi and
7 Mazzoncini, 2006; Mazzoncini *et al.*, 2010), in an area which since 1999 has been
8 managed according to the EU organic farming regulations (EC Reg. 1991/2092 and
9 2007/834). The MASCOT 5-yr crop rotation is maize, durum wheat (*Triticum durum*
10 Desf.), sunflower (*Helianthus annuus* L.), pigeon bean (*Vicia faba* L. var. minor), common
11 wheat (*Triticum aestivum* L.). Green manure, including hairy vetch, is grown in the
12 winter before maize and sunflower.

13 The experimental layout was a split-plot design with three replications, with a sub-plot
14 size of 8 × 8 m. The main plots were characterized by either the presence (GM+) or
15 absence (GM-) of a winter green manure.

16 Hairy vetch cv. Latigo was broadcast seeded at a rate of 100 kg ha⁻¹ on 15 September
17 2010 and 31 October 2011, in the first and second years, respectively. The relatively
18 high seeding rate (100 kg ha⁻¹) was chosen to promote an adequate above ground yield
19 biomass. No fertilization, crop protection or direct weeding measures were applied on
20 hairy vetch until termination, which was done by disc harrowing at the flowering stage
21 (8 April 2011 and 24 April 2012). Averaged across all plots, the biomass produced by
22 the vetch accounted for 1.2 (SD = 0.21) t ha⁻¹ of dry matter in 2011 and 5.3 (SD = 0.99) t
23 ha⁻¹ in 2012, corresponding to a N supply of 27.4 and 194 kg ha⁻¹ respectively. The sub-
24 plots included six different P fertilization (PF) treatments: an unfertilized control (C-);

1 rock phosphate (C⁺a); a green compost amended with rock phosphate powder at the
2 beginning of compost production, at a rate of 100 kg t⁻¹ fresh matter (EP); a compost
3 obtained from the same raw material as EP but not enriched in P (NEP); and rock
4 phosphate and a non P-enriched compost (C⁺b) before maize sowing. All the composts
5 were produced by the International Center for High Mediterranean Agronomic Studies
6 (CIHEAM – IAM, Bari, Italy) using the same raw material (clippings from lawns,
7 ornamental palms and olives) in EP, NEP and C⁺b in both years (Bustamante *et al.*, 2016;
8 Mihreteab *et al.*, 2016; Ciaccia *et al.*, 2017). Except the control (C⁻), all treatments
9 included an amount of compost or rock phosphate powder calculated to replenish the P
10 deficit of the five-year crop rotation, estimated as 24 kg ha⁻¹ (unpubl. obs.). Compost
11 treatment and rate of application are reported in Table 1.

12 Maize (cultivar PR36Y03, Pioneer FAO class 300) was sown on 18 April 2011 and 15
13 May 2012, respectively, in the first and second years, at a rate of 80,000 seeds ha⁻¹.
14 Maize phenology was assessed from the early stages to flowering using the BBCH scale
15 (Lancashire *et al.*, 1991). Most measurements were taken during the critical periods for
16 maize/weed competition, i.e. from 3- to 14-leaf stage according to Hall *et al.* (1992).
17 Maize plant height, phenological stage, number of leaves, mean leaf width, length and
18 area were measured on three plants plot⁻¹ 58 days after sowing (DAS) (BBCH scale from
19 31 to 37, mean = 34). In 2011 these data were used for a preliminary assessment of the
20 potential competitive ability of maize. In 2012, a higher number of measurements were
21 taken to obtain better estimates: plant height and phenological phase were assessed 24,
22 28, 32, 43, 50, 58, 64, 71 and 90 days after maize sowing, corresponding to mean BBCH
23 values of 15, 16, 18, 31, 32, 35, 52, 60, and 74 respectively. Number of leaves per plant,
24 mean leaf width, length and area, reported as Leaf Area Index (LAI), were measured 34

1 and 54 DAS. Crop N nutritional status was measured by a chlorophyll meter (SPAD-502,
2 Konica Minolta Holding, Inc.) at 24, 34, 43 and 50 DAS on the fourth and fifth leaves.
3 Total maize biomass was measured only at harvest in 2011, and at 28, 34, 43 DAS in
4 2012.

5 Weed density by species was measured before post-emergence inter-row cultivation
6 (hoeing), which happened only once in the two years when maize had five unfolded
7 leaves (BBCH 15). Weed density was assessed on three 50 × 50 cm sampling areas plot⁻¹
8 on 20 May 2011 and 8 June 2012, respectively. In 2011, weed biomass was very limited
9 throughout the maize cropping cycle due to drought and thus was not sampled. In 2012,
10 three weed biomass samplings were performed at 28, 34 and 43 DAS, respectively.

11

12 **Statistical analysis**

13 **Maize potential competitive ability**

14 Data from the preliminary study on the level of correlation among maize traits selected
15 as indicators of potential competitive ability (plant height, phenological stage, number of
16 leaves, mean leaf width, length and area) were analysed with a linear model to select
17 independent variables. For 2011 data, we used a split-plot ANOVA upon a Linear Mixed
18 Model with Gaussian distribution. Compost treatments, nested within green manure
19 treatments, were considered as a fixed factor and blocks as a random factor. Tukey's
20 post-hoc test was applied to cases that showed statistical significance. For 2012 data
21 (for which several parameters had repeated measures) time was added in the model as
22 a fixed effect (Faraway, 2005). Where more than one individual plant or plant organ
23 (leaves in the case of SPAD) were sampled, these sampling units were added to the
24 model as the nested random factor. For example, in the error structure of the model

1 concerning SPAD data, measured leaves (the fourth and fifth) were included as nested
2 effect within individual plants, and the three plants were included as nested effect
3 within each sampling area.

4 When ANOVA pre-assumptions were not met all data were appropriately transformed
5 (Gomez and Gomez, 1984) . Homoscedasticity and normality of residuals were checked
6 with the Bartlett test (Snedecor and Cochran, 1989) and the Shapiro test (Shapiro and
7 Wilk, 1965), respectively. Linear Mixed Model analyses were performed using the
8 “Lme4” package for R (Bates *et al.*, 2015). When transformation was ineffective in
9 meeting data requirements for ANOVA, a non-parametric Friedman test (Conover, 1980)
10 was used to highlight the factor(s) affecting the dependent variable, and the Conover
11 test (Conover, 1980) was used as post-hoc test.

12 In 2011, due to the severe drought, no weeds emerged in the third block, which was then
13 excluded from the analysis. To increase data accuracy, measurements were repeated
14 three times in each plot: these were considered as pseudo-replications and included in
15 the statistical model as a random effect nested within the block.

16

17 **Weed density and weed community diversity**

18 Using weed density data, the following parameters were calculated: species richness, the
19 Shannon diversity index (HS) (Magurran, 1988), the inverse Simpson index of diversity
20 (invsimp) (Peet, 1974), and Pielou's evenness index (J) (Sheldon, 1969). Weed density
21 was partitioned into functional groups upon the following response traits: (1) Raunkiær
22 life form for herbaceous species (Raunkiær, 1934), (2) Grime plant strategy (Grime,
23 1979), (3) two groups based on Ellenberg indicator values for soil fertility (N) (Pignatti
24 *et al.*, 2005): one group composed of species with higher value than the mean of all

1 species present, and the other composed of species with lower values than the mean. To
2 separate the effect of the tested factors on functional groups from their general effect on
3 overall weed density, we calculated the relative density by functional group (sum of the
4 densities of all species belonging to a functional group divided by total weed density,
5 expressed as a percentage). Species richness, diversity indices and relative density of
6 functional groups were considered as independent variables in a split-plot design
7 ANOVA, in a Mixed Effect Model, with a Poisson distribution for weed density and
8 species richness data, Gaussian distribution for HS, invsimps and J, and binomial
9 distribution for relative density by functional group. Tukey's post-hoc test was applied
10 where needed.

11

12 **Weed community composition**

13 Weed density data were also used to create a matrix of dissimilarities using the Bray-
14 Curtis dissimilarity index. A permutational multivariate analysis of variance was
15 performed to analyse if and how hairy vetch green manure and composts affected weed
16 community composition. With this analysis the distance matrix among sources of
17 variation can be partitioned and a linear model can be fitted to it. The significance of
18 each explanatory variable was obtained by means of *F*-tests based on sequential sums of
19 squares from permutations of the raw data, restricting permutations within each block
20 in order to take the sampling design into account.

21 The diversity matrix was also used for multivariate analysis through non-metric
22 multidimensional scaling (NMDS), which is considered the most robust unconstrained
23 ordination method in community ecology (Minchin, 1987). For each factor (green
24 manure or P fertilization) with a significant effect on weed species composition (in

1 terms of the results of the permutational multivariate analysis of variance), a scatter plot
2 based on samples was produced. Weed community analyses were carried out using the
3 “vegan” package for R (Oksanen *et al.*, 2009).

4

5 **Maize/weeds competitive interactions**

6 To understand whether green manure and/or P fertilization gave a competitive
7 advantage to maize or to weeds, a response comparison index (RCI) was computed
8 (Campiglia *et al.*, 2014). This index is based on the relative response index of weed
9 (RRI_w) and crop (RRI_c), calculated as indicated by Williams *et al.* (1998). RRI_w was
10 calculated as:

$$11 \quad RRI_w = (CBM_w - TBM_w) / (CBM_w + TBM_w)$$

12 where CBM_w is the weed above ground biomass in the control plot (C⁻ and GM⁻ for the P
13 fertilization and green manure effect, respectively), and TBM_w is the weed aboveground
14 biomass in every other treatment (EP, NEP, C⁺a and C⁺b for the P fertilization effect, and
15 GM⁺ for the green manure effect). RRI_w values < 0 mean that weed biomass is promoted
16 more by a given treatment than the control.

17 Similarly, RRI_c was computed as:

$$18 \quad RRI_c = (CBM_c - TBM_c) / (CBM_c + TBM_c)$$

19 where CBM_c and TBM_c are the crop total above ground biomass in the control and
20 treatment plots, respectively. RRI_c values < 0 mean that crop biomass is more stimulated
21 by a given treatment compared to the control. Finally, the Response Comparison Index
22 was calculated as:

$$23 \quad RCI = RRI_w - RRI_c$$

1 RCI gives an idea of crop/weed competitive interactions under each treatment. Positive
2 RCI values indicate that the crop is more competitive than weeds, whereas negative RCI
3 values indicate the opposite. Due to the lack of weed biomass data for 2011, RCI was
4 only computed on 2012 data at each sampling date (28, 34 and 43 DAS). When time had
5 a significant effect on the indices, the analyses were performed by sampling date. With
6 respect to the effects of green manure, due to the lower number of values produced by
7 the calculation procedure, time was added as a random factor in a Mixed Effect Model
8 with Gaussian distribution. A Z-test, using the BSDA package for R (Brill, 2005) was run
9 to assess whether RCIs were significantly higher than 0, and whether the RRIIs were
10 significantly different from 0.

11 Statistical analyses were carried out using R 3.0.3 (R Development Core Team, 2014)
12 with package lme4 (Bates *et al.*, 2015) for mixed models.

13

14 **Results and discussion**

15 **Maize potential competitive ability**

16 In 2011 and 2012 all maize leaf characteristics (number of leaves, mean leaf width,
17 length and area) were significantly ($P < 0.001$) and linearly correlated with leaf area
18 plant^{-1} (see Annex 2), hence only leaf area plant^{-1} results are shown.

19 According to the Friedman rank sum test, the phenological phase of maize in 2011 was
20 significantly ($P < 0.05$, Friedman's chi-squared statistic = 7.69) more advanced in plots
21 where green manure had been applied (median BBCH = 35) compared to plots where
22 GM was not applied (median BBCH = 33), while in 2012 no significant effect was
23 detected (see Annex 3). With respect to P application, no significant effects on crop
24 phenology were found in either year (data not shown). Leaf area plant^{-1} was significantly

1 increased by green manure application (GM⁺), and was 1.7-fold higher in 2011 at 58
2 DAS, and two- and 1.56-fold higher at 34 and 50 DAS, respectively, in 2012 (Table 2)
3 compared to GM⁻.

4 Regarding the P fertilization levels, only NEP in 2011 showed higher (+25%) values for
5 leaf area plant⁻¹ than the control (C⁻), while there was no significant effect in 2012. In
6 both years, green manure increased early stage plant height: in 2012 maize reached the
7 maximum plant height about 15 days earlier in green manured plots (Annex 3). Table 2
8 shows only plant height data for the period in which the green manure effect was
9 significant, i.e. between 43 (+35%) and 58 DAS (+29%). In 2011 maize plants were 17%
10 taller in NEP than in C⁻, while there was no evident P fertilization effect in 2012. There
11 was no interaction between green manure and P fertilization.

12 Chlorophyll content was affected only by green manure (P = 0.005), which led to an
13 average 28% increase in SPAD unit values compared to GM⁻ (time trends are shown in
14 Annex 3), an effect similar to that found by Radicetti *et al.* (2013). SPAD peak values
15 were observed between 30 and 40 DAS.

16 In 2012, total above ground maize biomass was significantly affected by time and by the
17 time × green manure interaction, therefore data were analyzed separately by date (i.e.,
18 28, 34 and 43 DAS). Green manure increased maize biomass across all sampling dates. In
19 fact the amount of biomass was two-fold, 2.57-fold and 2.46-fold higher than the no
20 green manure treatment at 28, 34 and 43 DAS respectively (Table 2). No significant
21 interaction between green manure and P fertilization was detected.

22 Among all maize parameters considered, the green manure application showed a clear
23 and consistent effect in enhancing plant height and LAI, which are two of the most
24 important traits expressing the potential crop competitive ability against weeds

1 (Mohammadi, 2007; Zystro *et al.*, 2012). There was a clear positive effect of green
2 manure in the first period of crop development, from 15 to 42 DAS, which is considered
3 to be within the critical period for crop/weed competition (Ferrero *et al.*, 1996). In
4 contrast, P-enriched compost did not have a clearly detectable effect on the potential
5 competitive ability of maize.

6

7 **Weed density, biomass and diversity**

8 Phosphorus fertilization ($P < 0.001$, Chi sq = 65.33, Df = 4) and green manure $\times$ P
9 fertilization interaction ($P < 0.01$, Chi sq = 33.36, Df = 4) significantly affected total weed
10 density in 2011, but not in 2012. In 2011, the differences in total weed density among P
11 fertilization treatments were much lower in GM+ than in GM- plots. In the GM- plots, C+b
12 had 31.5% lower weed density than C-, whereas NEP and C+a had 15.1% and 15.5%
13 higher weed density respectively (Fig. 1). Legume green manures have been reported to
14 increase weed emergence (Blum, 1997; Ciaccia *et al.*, 2015), but not in our study.
15 However, evidence of reduced weed biomass as a result of hairy vetch incorporation in
16 soil was also scarce, and was limited to 28 DAS in 2012 (1.4 vs 4.8 g m⁻² in the GM+ and
17 GM- plots, respectively). At subsequent sampling dates, total weed biomass was similar
18 and rather low (on average 0.6 g m⁻² at 34 DAS and 0.4 g m⁻² at 43 DAS). Neither P
19 fertilization nor the green manure $\times$ P fertilization interaction had a significant effect on
20 weed biomass. The weed biomass reduction observed at 28 DAS in GM+ plots may have
21 been due to an allelopathic effect produced by *V. villosa*, as reported by Bittencourt *et al.*
22 (2013).

23 The number of species (S) and the Shannon index (H') were not affected by green
24 manure or P fertilization in either year (average S: 4.6 in 2011 and 7.0 in 2012; average

1 H': 1.85 in 2011 and 1.57 in 2012). On the other hand, the Inverse Simpson index was
2 significantly higher ($P < 0.05$) in plots where green manure was incorporated into the soil
3 (1.30) than in those without green manure (1.25). Comparing the results of Shannon and
4 Inverse Simpson indices, as suggested by Morris *et al.* (2014), reveals that the weed
5 community diversity was mainly driven by common species in the first year and by rare
6 species in the second year. Results for N and P dynamics (especially in 2012) showed
7 higher values in GM+ at early growth stages (Ciaccia, 2014). Despite these significant
8 differences observed, which are considered among the main levers for change in weed
9 diversity (Hawes *et al.*, 2010 for N; Wassen *et al.*, 2005 for P), the practices tested in our
10 study did not deplete weed community diversity. In fact, the only significant effect
11 exerted by green manure (in only one year) was to increase the diversity of common
12 weed species, shifting the weed community to a more balanced composition (i.e., with a
13 high number of evenly distributed species; Bàrberi, 2002). A more diverse weed flora is
14 less competitive with the cash crop, and can minimize the risks of predominance of a
15 few competitive species, which occupy specific ecological niches, by competing with the
16 cash crops for the same resources (Poffenbarger *et al.*, 2015; Storkey and Neve, 2018).

17

18 **Weed community composition**

19 According to the results of the permutational multivariate analysis of variance (Table 3),
20 green manure was the only factor that significantly affected weed community
21 composition at an early stage in both years ($P < 0.01$), the magnitude of the effect was
22 higher in 2012 than in 2011. Not surprisingly, P application had little effect on weed
23 community composition at that stage, since P availability did not differ among the
24 several P fertilization treatments until stem elongation (Ciaccia, 2014).

1 NMDS highlighted a clear green manure effect on weed assemblages in both years
2 (Figures 2a and 2b). Species affinity with green manure application was consistent
3 between years (compare Figures 2a and 2b). In particular, green manure selected for
4 annual nitrophilous dicotyledonous species (the Ellenberg N values for each species
5 sampled are reported in Annex 4) such as *Anagallis arvensis* L. in 2011 and *Stellaria*
6 *media* (L.) Vill. in 2012, whereas it selected against annual grasses such as *Lolium*
7 *multiflorum* Lam. and perennial species such as *Cyperus esculentus* L. Competition for
8 resources exerted by the presence of hairy vetch likely reduced the possibility of
9 accumulating photosynthates in underground storage organs in species (e.g. perennials)
10 for which this represents a key survival strategy.

11

12 **Weed community functional analysis**

13 In accordance with the Raunkiær life form methodology, we classified the observed
14 species into the three categories of therophytes (i.e., mostly annual plants that
15 reproduce by seeds), geophytes (i.e., perennial plants that resprout from underground
16 vegetative organs), and hemicryptophytes (i.e., plants that resprout from buds placed at
17 a soil level).

18 In 2011, the relative density of the Raunkiær life forms was never significantly affected
19 by the experimental factors. In 2012, due to a very high incidence of therophytes (in
20 75.6% of the samples, the only species present were therophytes), no such analysis was
21 performed. Regarding Grime's plant strategy groups, the relative density of ruderals (R)
22 was 100% and 42% higher in green manured plots in 2011 and 2012 respectively than
23 in plots where green manure was not applied. Similarly, competitive-ruderals (CRs)
24 were favored by hairy vetch incorporation in 2012 (relative density: 39.8%) (Table 4).

1 Species with a high affinity for rich soils (i.e. Ellenberg indicator values for soil fertility >
2 5, see Pignatti *et al.*, 2005 and list in Annex 4) were not significantly affected by green
3 manure or compost type in either years (data not shown), despite the higher levels of
4 soil N found after green manure incorporation (Ciaccia, 2014).

5 Our study suggests that P application, in the form of P-enriched compost, does not affect
6 the magnitude of weed emergence, unlike Blackshaw and Molnar (2009) who found a
7 significant effect of P application. However, these authors did not use enriched compost
8 as P source.

9 On the other hand ruderal and competitive-ruderal species seem to find more favorable
10 conditions after green manure application. Farmers should thus monitor the abundance
11 of such species in subsequent spring-sown crops and use appropriate control strategies
12 when necessary. This would be particularly important in case of high presence of weed
13 species emerging at the same time as maize, thus potentially more competitive (e.g.
14 *Xanthium strumarium* L., *Datura stramonium* L., *Echinochloa crus-galli* L., *A. retroflexus*,
15 *Sorghum halepense* L., *Chenopodium album* L.). This could lead to a potentially high yield
16 reduction (Gołębiowska and Kieloch, 2016; Yousefi *et al.*, 2015).

17

18 **Maize/weed competition**

19 When considering the effect of green manure on weed and crop competition, neither the
20 RRI or RCI indices showed any significant interaction between green manure and P
21 fertilization (Chi-square:1.446 and 2.515 for RRI_c in 2011 and 2012; 1,102 for RRI_w in
22 2012, and 1.562 for RCI in 2012). In both years, the results of the Z-test on RRI clearly
23 indicated a competitive advantage for maize when grown after green manure ($RRI_c < 0$),
24 while weeds (in 2012) were not favored by green manure application (RRI_w not

1 significantly higher or lower than 0), as reported in Table 5. As a consequence, the RCI
2 for the green manure factor showed that crop/weed competitive relationships favored
3 the crop to the detriment of weeds when green manure was applied.

4 This result has very important practical implications and is consistent with Liebman and
5 Davis (2000), who found that the competitive ability of the cash crop was enhanced by
6 the application of leguminous green manure. Although we did not measure the N
7 content in weeds, we can hypothesize that the high soil N availability and N uptake by
8 maize during the critical period for crop/weed competition (Ciaccia *et al.*, 2017),
9 following green manure incorporation, played a key role in shifting the competitive
10 balance towards the crop. As shown in Table 2, maize traits related to competitive
11 ability (plant height, leaf area) had higher values in green manured crops, an effect most
12 likely driven by higher N availability.

13 The RRI and RCI indices that were calculated to study the effect of the P fertilization
14 factor on crop/weed competition (Table 6) also indicated a competitive advantage for
15 the cash crop (negative RRI_c and positive RRI_w) when P was applied. However, the way P
16 was applied did not significantly affect the results (Chi-square: 0.655 and 3.876 for RRI_c
17 in 2011 and 2012; 7.211, 8.039 and 1.442 for RRI_w at 28, 34 and 43 DAS respectively in
18 2012; 2.535, 9.414 and 0.447 for RCI at 28, 34 and 43 DAS respectively in 2012). In
19 contrast, RRI_w and RCI calculated at 34 DAS in 2012 revealed that weeds were more
20 competitive when P was applied together with green manure, and that maize was more
21 competitive when P was applied alone (Table 6). Although this effect was found just in
22 one of the three sampling dates, when N and P are more available for weeds they may
23 stimulate weed growth even in a quite N-demanding crop like maize (in accordance with
24 Di Tomaso, 1995 and Davis and Liebman, 2001). However, in our study the overall effect

1 of green manure and P fertilization was to increase the competitive ability of maize
2 against weeds.

3

4 **Conclusions and recommendations**

5 The data presented in this paper confirm that the complexity of interactions between
6 soil fertility, crop and weeds can be steered towards a crop benefit by implementing
7 agronomic techniques that increase soil fertility whilst keeping weeds under control.
8 Combining high N₂-fixing legume green manures with P-enriched amendments can
9 promote nutrient cycling in organic stockless cropping systems, where the absence of
10 farmyard manure may lead to a shortage of soil N and P in the long term. Nevertheless, in
11 our conditions there was no immediate effect of P-enriched compost on early maize
12 growth. Likewise, the application of green manure and/or P enriched compost did not
13 increase early stage weed infestation in maize. We argue that the dry conditions might
14 have indeed slowed down the mineralization rate of the green manure and of the
15 composts, leading to no immediate availability of N, and especially P, for either maize or
16 weed plants. Further research under more humid spring conditions is thus required to
17 unravel the potential of these two nutrient sources of suppressing weeds in maize. Soil
18 incorporation of a hairy vetch green manure clearly enhanced the expression of
19 competitive traits of maize (i.e., plant height and LAI) and, overall, the competitive ability
20 of maize against weeds. On the other hand, green manure shifted weed community
21 composition towards a higher relative abundance of ruderal and competitive-ruderal
22 species. These dynamics should be monitored if these species become dominant, in
23 which case dedicated management tactics would need to be implemented. We found that
24 hairy vetch green manure reduced the development of weed species groups such as

1 creepers and perennials (e.g. *C. esculentum*), which are often detrimental to maize
2 production. Overall, the weed community shifts induced by green manure seem to lead
3 to a less aggressive weed community for subsequent spring-summer crops. However this
4 needs to be confirmed in a longer-term perspective, in particular considering the very
5 dry conditions of the two years studied during our tests.

6

7 **Acknowledgements**

8 This study was part of the COMPARABIMUS project, funded by the Italian Ministry of
9 Agricultural, Food and Forestry Policies (MiPAAF). The authors wish to thank Giacomo
10 Nardi and the CIRAA staff for their technical support.

1 **Reference**

- 2 Anugroho, F., Kitou, M., Nagumo, F., Kinjo, K., and Tokashiki, Y. 2009. Growth, nitrogen
3 fixation, and nutrient uptake of hairy vetch as a cover crop in a subtropical
4 region. *Weed Biology and Mangement* 9(1): 63-71.
- 5 Bàrberi, P. 2002. Weed management in organic agriculture: are we addressing the right
6 issues? *Weed Research* 42(3): 177-193.
- 7 Bàrberi, P., and Mazzoncini, M. 2001. Changes in weed community composition as
8 influenced by cover crop and management system in continuous corn. *Weed*
9 *Science* 49(4): 491-499.
- 10 Bàrberi, P., and Mazzoncini, M. 2006. The MASCOT (Mediterranean Arable Systems
11 Comparison Trial) long-term experiment (Pisa, Italy). Long-term Field
12 experiments in Organic Farming. *ISO FAR Sci. Series* (1): 1-15.
- 13 Bates, D., Maechler, M., Bolker, B. and Walker, S. 2015. Fitting Linear Mixed-Effects
14 Models Using lme4. *Journal of Statistical Software* 67(1): 1-48.
- 15 Bittencourt, H. V. H., Lovato, P. E., Comin, J. J., Lana, M. A., Altieri, M. A., Costa, M. D., and
16 Gomes, J. C. 2013. Effect of winter cover crop biomass on summer weed
17 emergence and biomass production. *Journal of Plant Protection Research* 53(3):
18 248-252.
- 19 Blackshaw, R. E. and Brandt R. N. 2008. Nitrogen fertilizer rate effects on weed
20 competitiveness is species dependent. *Weed Science* 56(5): 743-747.
- 21 Blackshaw, R. E., and Molnar, L. J. 2009. Phosphorus fertilizer application method affects
22 weed growth and competition with wheat. *Weed Science* 57(3): 311-318.

- 1 Blackshaw, R. E., and Brandt, R. N. (2009). Phosphorus fertilizer effects on the
2 competition between wheat and several weed species. *Weed Biology and*
3 *Management* 9(1): 46-53.
- 4 Blackshaw, R. E., Brandt, R. N., Henry Janzen, H., and Entz, T. 2004. Weed species
5 response to phosphorus fertilization. *Weed Science*, 52(3): 406-412.
- 6 Blum, U., King, L. D., Gerig, T. M., Lehman, M. E., and Worsham, A. D. 1997. Effects of
7 clover and small grain cover crops and tillage techniques on seedling emergence
8 of some dicotyledonous weed species. *American Journal of Alternative*
9 *Agriculture* 12(4): 146-161.
- 10 Brandsæter, L. O., Heggen, H., Riley, H., Stubhaug, E., and Henriksen, T. M. 2008. Winter
11 survival, biomass accumulation and N mineralization of winter annual and
12 biennial legumes sown at various times of year in Northern Temperate Regions.
13 *European Journal of Agronomy* 28(3): 437-448.
- 14 Brill, R. V. 2005. *Basic Statistics and Data Analysis*. Technometrics 47(1). CA USA.
- 15 Bustamante, M. A., Ceglie, F. G., Aly, A., Mihreteab, H. T., Ciaccia, C., and Tittarelli F. 2016.
16 Phosphorus availability from rock phosphate: Combined effect of green waste
17 composting and sulfur addition. *Journal of Environmental Management* 182: 557-
18 563.
- 19 Campiglia, E., Radicetti, E., Brunetti, P., and Mancinelli, R. 2014. Do cover crop species
20 and residue management play a leading role in pepper productivity? *Scientia*
21 *Horticulturae*, 166: 97-104.
- 22 Ciaccia, C. 2014. Effetto combinato del sovescio e del compost sulla dinamica di azoto e
23 fosforo in sistemi colturali arativi biologici senza zootecnia (stockless). PhD

1 Thesis, Retrieved from ETD database number: 07232014-143443, University of
2 Pisa.

3 Ciaccia, C., Ceglie, F., Tittarelli, F., Antichi, D., Carlesi, S., Testani, E., and Canali, S. 2017.
4 Green manure and compost effects on NP dynamics in Mediterranean organic
5 stockless systems. *Journal of Soil Science and Plant Nutrition*, 17(3): 751-769.

6 Ciaccia, C., Montemurro, F., Campanelli, G., Diacono, M., Fiore, A., and Canali, S. 2015.
7 Legume cover crop management and organic amendments application: Effects on
8 organic zucchini performance and weed competition. *Scientia Horticulturae*, 185:
9 48-58.

10 Conover, W. J. 1980. *Practical nonparametric statistics*,. New York, USA.

11 Davis, S.A. and Liebman, M., 2001. Nitrogen source influence wild mustard growth and
12 competitive effect on sweet corn. *Weed Science* 49 (4): 558–566.

13 Di Tomaso, J.M. 1995. Approaches for improving crop competitiveness through the
14 manipulation of fertilization strategies. *Weed Science* 43 (3): 491–497.

15 Faraway, J. J, 2005. *Extending the linear model with R: generalized linear, mixed effects*
16 *and nonparametric regression models*. Boca Raton, Florida: CRC press.

17 Ferrero, A., Scanzio, M., and Acutis, M. 1996. Critical period of weed interference in
18 maize. *Proceedings of the Second International Weed Control Congress*.
19 Copenhagen, Denmark:Department of Weed Control and Pesticide Ecology: 171-
20 176.

21 Gomez, K. A., and Gomez A. A. 1984. *Statistical procedures for agricultural research*. New
22 York, USA: John Wiley & Sons.

23 Grime, J. P. 1979. *Plant strategies and vegetation processes*. Chichester, New York,
24 Brisbane, Toronto: John Wiley & Sons.

- 1 Grant, C. A., Flaten, D. N., Tomaszewicz, D. J., and Sheppard, S. C. 2001. The importance of
2 early season phosphorus nutrition. *Canadian Journal of Plant Science* 81 (2): 211-
3 224.
- 4 Hall, M. R., Swanton, C. J., and Anderson, G. W. 1992. The critical period of weed control
5 in grain corn (*Zea mays*). *Weed Science* 40 (3): 441-447.
- 6 Hartwig, N. L., and Ammon, H. U. 2002. Cover crops and living mulches. *Weed Science* 50
7 (6): 688-699.
- 8 Hawes, C., Squire, G. R., Hallett, P. D., Watson, C. A., and Young, M. 2010. Arable plant
9 communities as indicators of farming practice. *Agriculture, Ecosystems &*
10 *Environment* 138 (1): 17-26.
- 11 Hoveland, C. S., Buchanan, G. A., and Harris, M. C. 1976. Response of weeds to soil
12 phosphorus and potassium. *Weed Science* 24 (2): 194-201.
- 13 Karasawa, T., and Takahashi, S. 2015. Introduction of various cover crop species to
14 improve soil biological P parameters and P uptake of the following crops.
15 *Nutrient Cycling in Agroecosystems* 103 (1): 15-28.
- 16 Lancashire, P. D., Bleiholder, H., Boom, T. V. D., Langelüddeke, P., Stauss, R., Weber, E.,
17 and Witzemberger, A. 1991. A uniform decimal code for growth stages of crops
18 and weeds. *Annals of Applied Biology* 119 (3): 561-601.
- 19 Lehoczky, É., Gólya, G., Tamás, J., and Németh, T. 2015. Biodiversity and Biomass
20 Production of Weeds in a Long-Term Fertilization Experiment. *Communications*
21 *in Soil Science and Plant Analysis* 46 (Suppl. 1): 390-398.
- 22 Liebman, M., and Davis A. S. 2000. Integration of soil, crop and weed management in
23 low-external-input farming systems. *Weed Research*, 40 (1): 27-48.

- 1 Magurran, A.E. 1988. Ecological Diversity and its Measurement. Princeton University
2 Press, Princeton.
- 3 Mazzoncini, M., Canali, S., Giovannetti, M., Castagnoli, M., Tittarelli, F., Antichi, D.,
4 Nannelli, R., Cristani, C., and Bàrberi, P. 2010. Comparison of organic and
5 conventional stockless arable systems: A multidisciplinary approach to soil
6 quality evaluation. *Applied Soil Ecology* 44 (2): 124-132.
- 7 Mihreteab, H. T., Ceglie, F. G., Aly, A., and Tittarelli, F. 2016 Rock phosphate enriched
8 compost as a growth media component for organic tomato (*Solanum*
9 *lycopersicum* L.) seedlings production. *Biological Agriculture and Horticulture* 32
10 (1): 7-20.
- 11 Minchin, P. R. 1987. An evaluation of the relative robustness of techniques for ecological
12 ordination. In: *Theory and Models in Vegetation Science*: 89-107. Springer
13 Netherlands.
- 14 Mohammadi, G. R. 2007. Growth parameters enhancing the competitive ability of corn
15 (*Zea mays* L.) against weeds. *Weed Biology and Management*, 7 (4): 232-236.
- 16 Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., Meiners, T.,
17 Muller, C., Obermaier, E., Prati, D., and Rillig, M. C. 2014. Choosing and using
18 diversity indices: insights for ecological applications from the German
19 Biodiversity Exploratories. *Ecology and Evolution*, 4 (18): 3514-3524.
- 20 Njeru, E. M., Avio, L., Sbrana, C., Turrini, A., Bocci, G., Bàrberi, P., and Giovannetti, M.
21 2014. First evidence for a major cover crop effect on arbuscular mycorrhizal
22 fungi and organic maize growth. *Agronomy for Sustainable Development* 34 (4):
23 841-848.

- 1 Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H.,
2 and Wagner, H. 2009. Vegan: community ecology package. R package version
3 1.15-2. R Foundation for Statistical Computing, Vienna, Austria.
- 4 Owla, M. L., Nepalia, V., Chouhan, G. S., and Singh, D. 2015. Effect of fertility levels,
5 nutrient sources and weed control on weed dynamics and yield of quality protein
6 maize (*Zea mays*) and relative nitrogen and phosphorus uptake. Indian Journal of
7 Agronomy, 60 (2): 267-272.
- 8 Peet, R. K. 1974. The Measurement of Species Diversity. Annual Review of Ecology and
9 Systematics 5: 285-307.
- 10 Pignatti, S., Menegoni, P., and Pietrosanti, S. 2005. Bioindicator values of vascular plants
11 of the Flora of Italy. Braun-Blanquetia 39: 3-95.
- 12 R Development Core Team 2014. R: A language and environment for statistical
13 computing. R Foundation for Statistical Computing, Vienna, Austria. URL
14 <http://www.R-project.org/>.
- 15 Radicetti, E., Mancinelli, R., and Campiglia, E. 2013. Influence of winter cover crop
16 residue management on weeds and yield in pepper (*Capsicum annuum* L.) in a
17 Mediterranean environment. Crop Protection 52 (10): 64-71.
- 18 Rajcan, I., and Swanton, C. J. 2001. Understanding maize–weed competition: resource
19 competition, light quality and the whole plant. Field Crops Research 71 (2): 139-
20 150.
- 21 Raunkiaer, C. 1934. The life forms of plants and statistical plant geography; being the
22 collected papers of C. Raunkiaer. Clarendon Press, Oxford.
- 23 Reddy, K. N., and Koger, C. H. 2004. Live and killed hairy vetch cover crop effects on
24 weeds and yield in glyphosate-resistant corn. Weed Technology 18 (3): 835-840.

- 1 Rotchés-Ribalta, R., Armengot, L., Mäder, P., Mayer, J., and Sans, F. X. 2017. Long-term
2 management affects the community composition of arable soil seedbanks. *Weed*
3 *Science*, 65 (1): 73-82.
- 4 Shapiro, S. S, and Wilk, M. B. 1965. An analysis of variance test for normality (complete
5 samples). *Biometrika* 52 (3 and 4): 591-611.
- 6 Sheldon, A. L. 1969. Equitability indices: dependence on the species count. *Ecology* 50
7 (3): 466-467.
- 8 Snedecor, G. W. and Cochran, W. G. 1989. *Statistical Methods*, Eighth Edition, Iowa State
9 University Press.
- 10 Storkey, J., and Neve, P. 2018. What good is weed diversity?. *Weed Research*, 58 (4): 239-
11 243.
- 12 Sweeney, A. E., Renner, K. A., Laboski, C., and Davis, A. 2008. Effect of fertilizer nitrogen
13 on weed emergence and growth. *Weed Science*, 56 (5): 714-721.
- 14 Tang, L., Cheng, C., Wan, K., Li, R., Wang, D., Tao, Y., Pan, J., Xie, J., and Chen, F. 2014.
15 Impact of fertilizing pattern on the biodiversity of a weed community and wheat
16 growth. *Plos One*, 9 (1): e84370.
- 17 Thomas, P. E. L., and Allison, J. C. S. 1975. Competition between maize and *Rottboellia*
18 *exaltata*. *The Journal of Agricultural Science*, 84 (2): 305-312.
- 19 Tollenaar, M., Nissanka, S. P., Aguilera, A., Weise, S. F. and Swanton, C. J. 1994. Effect of
20 weed interference and soil nitrogen on four maize hybrids. *Agronomy Journal*, 86
21 (4): 596-601.
- 22 Wassen, M. J., Venterink, H. O., Lapshina, E. D., and Tanneberger, F. 2005. Endangered
23 plants persist under phosphorus limitation. *Nature* 437 (7058): 547-550.

- 1 Williams, M. M., Mortensen, D. A., and Doran, J. W. 1998. Assessment of weed and crop
2 fitness in cover crop residues for integrated weed management. *Weed Science* 46
3 (5): 595-603.
- 4 Zystro, J. P., de Leon, N., and Tracy, W. F. 2012. Analysis of traits related to weed
5 competitiveness in sweet corn (*Zea mays* L.). *Sustainability* 4 (4): 543-560.

1 Figures captions:

2 Figure1: Total weed density as compost x green manure (GM) interaction; error bars
3 represent the standard error (SE). GM presence is reported in grey, GM absence
4 in black. (C-, untreated -no compost, no rock phosphate-; C+a, rock phosphate
5 only; C+b, Compost + rock phosphate; EP, P enriched compost; NEP, compost
6 only; composition and rate of application are reported in Table1).

7 Figure 2a: Site ordination (NMDS) based on floristic similarities of 20 plots (k=2, non-
8 metric fit: $R^2=0.953$, stress=0.215). GM, Green manure. Species names are
9 reported in Annex 4.

10 Figure 2b. Site ordination (NMDS) based on floristic similarities of 30 plots (k=2, non-
11 metric fit: $R^2=0.939$, Stress: 0.246).GM, Green manure. Species names are
12 reported in Annex 4.

Dear Editor,

I am re-Submitting the manuscript "Green manure and phosphorus fertilization affect weed community composition and crop/weed competition in organic maize" hoping the new version meets your and reviewers expectation, to be considered suitable for publication in Renewable Agriculture and Food Systems. We have deeply reviewed the writing, as suggested, and we also asked a native professional English, Adrian Wallwork, to edit and proofread the English of the paper.

In organic stockless condition long term sustainability of soil fertility is often a challenge. Many different techniques are available for farmer to manage carbon, nitrogen and phosphorus cycle. In this article we analyze how an innovative technique (application of different compost enriched in phosphorus) and more common technique (green manure rich in nitrogen application), may also affect a key element of the agroecosystem: weed/crop competition. The approach we used is that techniques thought to manage soil fertility, can also be considered in a wide strategy to manage weeds. Two dry seasons of maize crop grown in stockless rainfed condition were studied analyzing weed pressure, species composition and competition on the main crop. Since the elements studied are quite wide we add some extra material as an annex. Images are available in both format, pdf and tiff, so both the file types were attached. We are also uploading a document containing all the answers to the reviewers note and the old manuscript with the track changes, so all the change can be clearly visible.

The reviewers we suggested were:

Robert E. Blackshaw, Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, AB T1J 4B1, Canada; blackshaw@agr.gc.ca

Randall N. Brandt, Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, AB T1J 4B1, Canada; randall@agr.gc.ca

Emanuele Radicetti, Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia, Università della Tuscia, via S. Camillo de Lellis, 01100 Viterbo, Italy
radicetti@unitus.it

Ennio Campiglia, Università degli Studi della Tuscia Viterbo, Department of Agricultural and Forestry Sciences, Viterbo, Italy
campiglia@unitus.it

I hope you could enjoy reading this new version of the manuscript. On behalf of my co-authors, I declare that this is an original manuscript that has not submitted to any other journal.

Best Regards,
the corresponding author

Decision for RAFS-D-18-00135R1

Editorial Office
Renewable Agriculture and Food Systems

Comments to the author:

Dear Author (cc to Stefano Canali and Fabio Tittarelli):

Although the changes you have made to the document regarding the scientific portions of the paper are acceptable, unfortunately the writing is still problematic. Our editors do not have a time to go line by line to revise the document as needed. In some areas, there are still issues with the English language. In others, there are problems with expanded/substantiated ideas, and in others it is poor sentence and paragraph structure. We will not be able to accept this paper until the writing has been improved. We have cced Stefano and Fabio on this letter to see if there is some assistance they can provide you.

Thank you again for submitting your article. Editor-in-Chief office

Reply: We have deeply reviewed the writing, as suggested, and we also asked a native professional English, Adrian Wallwork, to edit and proofread the English of the paper.

A track changes version of the paper is also provided ("track changes post 9th Jan.doc").

We hope the quality of the written English meets now your expectations.

Best regards
Stefano Carlesi

1 **Green manure and phosphorus fertilization affect weed community composition**
2 **and crop/weed competition in organic maize**

3
4 **Keywords:** hairy vetch, compost, soil amendment, Integrated Weed Management, weed
5 diversity

6
7 **Abstract**

8 Green manure and compost enriched in phosphorus can promote the sustainability of
9 cropping systems by increasing soil fertility over the long term. They can also be used to
10 manage crop/weed interactions, a key element in guaranteeing ~~an appropriate proper~~
11 level of satisfactory crop yields. We studied how green manuring with hairy vetch (*Vicia*
12 *villosa* Roth.) and the application of different types of phosphorous-enriched compost
13 affect weed/maize (*Zea mays* L.) interactions in an organic stockless Mediterranean
14 agroecosystem for two consecutive dry years. Green manure stimulated the expression
15 of maize traits related to a higher competitive ability against weeds, such as early
16 growth, height and leaf area index, while the effect of compost was less clear. Regarding
17 crop/weed competition, both green manuring and a phosphorus enriched compost
18 application gave a significant advantage to maize. Neither green manure nor ~~the~~
19 compost increased total weed density and biomass compared to the control. Green
20 manuring, significantly affected the weed community composition. The relative density
21 of ruderal and competitive-ruderal species (according to Grime's classification) was
22 higher in plots where the green manure was applied. The use of green manure, together
23 with novel composting techniques, significantly affected crop/weed competitive
24 interactions, favouring maize, but also creating favourable conditions for unwanted

Style Definition: Heading: Font: (Default) Arial, 14 pt, Space Before: 12 pt, After: 6 pt, Keep with next

Style Definition: Text body: Space After: 6 pt

Style Definition: Index: Suppress line numbers

Style Definition: Comment Text: (Asian) Chinese (PRC), Kern at 1.5 pt, Line spacing: Double, Don't adjust space between Asian text and numbers, Font Alignment: Baseline

Style Definition: Preformatted Text: Font: (Default) Courier New, 10 pt

Style Definition: Table Contents: Suppress line numbers

Style Definition: Table Heading: Font: Bold, Centered

1 weed species such as competitive-ruderals. Increasing nitrogen availability in the early
2 growth stages of maize through green manuring can increase crop competitive ability.
3 However, ~~but~~ this may not suffice to preserve the system from future weed problems,
4 should potentially detrimental species be selected. Dedicated strategies for the control
5 of emerging weed species may thus be needed.

6

7 **Word count :**

8 **Abstract:** ~~255254~~

9 **Main text** 5263

10 **References:** ~~17071646~~

11

12 **Introduction**

13 The presence of weeds throughout the cropping cycle of maize (*Zea mays* L.) can reduce
14 the crop yield in many different ways, for example by competing for water, mineral
15 nutrients and light (Rajcan and Swanton, 2001). Competition is important especially at
16 early crop development stages, but may also have consequences at later stages. For
17 instance, Tollenaar *et al.* (1994) reported reduced ear leaf chlorophyll concentration at
18 the silking stage of maize grown under early high weed pressure compared to weed-free
19 maize. Thomas and Allison (1975) also observed lower maize root development with
20 weed interference compared to weed-free conditions.

21 In organic farming, weed control in maize can be extremely challenging and relies on an
22 Integrated Weed Management (IWM) strategy through the application of both direct
23 (e.g. mechanical tools or flaming) and indirect methods (Bàrberi, 2002). Indirect
24 methods include all crop practices that prevent high weed occurrence while

1 simultaneously stimulating crop growth and competitive ability. In this context, nitrogen
2 (N) and phosphorus (P) fertilization may play a crucial role (Rajcan and Swanton, 2001;
3 Grant *et al.*, 2001). In fact, crop management recommendations rarely consider that
4 added nutrients might enhance weed growth as well as crop growth, resulting in
5 crop/weed competitive interactions which are potentially detrimental for the crop yield
6 (Blackshaw *et al.*, 2004; Blackshaw and Brandt, 2008). Information on how each
7 nutrient type affects crop/weed competition might help to improve IWM systems. N and
8 P availability may affect the maize capacity to compete with weeds, by favouring the
9 expression of plant traits that are more related to a higher competitive ability, e.g. leaf
10 area and plant height. Mohammadi (2007) showed that leaf area is one of the most
11 reliable parameters to estimate maize competitive ability against weeds, while Zystro *et al.*
12 (2012) found that plant height was the best predictor for estimating the weed
13 suppressive ability of sweet maize varieties.

14 In organic stockless cropping systems, where it is not possible to amend the soil with
15 raw animal effluents or farmyard manure, N and P could be supplied by soil
16 incorporation of green manures, composts, and natural fertilizers such as rock
17 phosphates.

18 Weed suppression and nutrient (especially N) release are two of the many benefits
19 potentially provided by green manures. Legume green manures, such as hairy vetch
20 (*Vicia villosa* Roth.), supply N to the soil through symbiotic N₂-fixation and increase soil
21 P availability by releasing acid root exudates (Karasawa and Takahashi, 2015) and/or
22 hosting symbioses with arbuscular ~~mycorrhizal~~ ~~micorrhizal~~ fungi (AMF) (Njeru *et al.*,
23 2014). Hairy vetch is one of the most efficient N-fixing crops (e.g. Hartwig and Ammon,
24 2002), which accumulates a large amount of N during its growing cycle (Anugroho *et al.*,

Formatted: Font: Italic

1 2009).About 50% of this N is readily available for the subsequent cash crop after soil
2 incorporation (green manuring) (Brandsæter *et al.*, 2008), ~~but~~ also for the field weed
3 community (Bittencourt *et al.*, 2013).

4 While the effect of fertilization on weed composition has been investigated in several
5 studies (Tang, *et al.*, 2014), little information is available on the effect of hairy vetch
6 green manure on weed community composition in the subsequent cash crop. Legume
7 green manures can change weed communities thanks to the release of allelopathic
8 compounds (Bittencourt *et al.*, 2013) and by reducing the amount of resources available
9 to weeds during the legume green manure growing season (Reddy and Koger, 2004).
10 Weed seedling emergence and biomass after soil incorporation of a legume cover crop
11 can be affected by the availability of residual N mineralized from the cover crop biomass,
12 although this effect varies according to the weed species (Sweeney *et al.*, 2008).

13 Soil P levels may affect weed as well as crop growth, ~~thus~~ resulting crop/weed
14 competitive interactions may be influenced by P management (Blackshaw and Brandt,
15 2009). Nitrogen and phosphorus are absorbed throughout a plant's growing cycle, but
16 the initial stages of development are the most critical. The availability of N is crucial in
17 sustaining maize yield and should be provided at all growing stages, including the grain
18 filling periods, especially for late season genotypes grown under wet conditions (Rajcan
19 and Swanton, 2001). In dry climates, N availability at early stages (from emergence to 5-
20 6 leave stage) is a key in supporting maize growth, as it can counteract the negative
21 effects of drought (de Oliveira *et al.*, 2018). For P, availability in the soil between the
22 maize sowing and ~~six-leafsix-leave~~ stage is fundamental for a good establishment of the
23 crop, as P deficiency can slow down the appearance of new leaves (Grant *et al.*, 2001).

Formatted: Font: Italic

1 Weed species vary considerably in their ability as P scavengers (Blackshaw *et al.*, 2004).
2 Weed biomass responds positively to increasing amounts of soil P (Hoveland *et al.*,
3 1976), but the magnitude of responses varies markedly across species (Lehoczky *et al.*,
4 2015; Owla *et al.*, 2015). Blackshaw *et al.* (2004) found that P fertilization can have a
5 large impact on weed growth and that soil P levels affect crop/weed competitive
6 ~~interactions. In particular, interactions, given that~~ weeds ~~also~~ seem more sensitive to low
7 P and K levels than crop species (Hoveland *et al.*, 1976). Despite studies on the potential
8 influence of P on weed populations and crop/weed competitive relationships, there is a
9 lack of information on how weed species respond to organic sources of P (e.g. composts
10 naturally rich in P or enriched with high P-content materials) and on the combined use
11 of such composts with green manure.
12 We conducted a field experiment on a long-term stockless rainfed arable crop rotation.
13 The aim was to investigate the effect of the combined use of a hairy vetch cover crop as
14 winter green manure with different types of composts on weed suppression and weed
15 community composition in a subsequent maize crop. We wanted to verify whether the
16 combined use of hairy vetch and P-enriched compost: (i) enhances the competitive
17 ability of organic maize by supplying the crop with sufficient levels of N and P and by
18 sustaining crop growth especially at early stages; (ii) reduces weed competitiveness by
19 reducing weed emergence and (iii) affects weed species composition.-

20

21 **Materials and methods**

22 To test our three hypotheses, a field trial was established at the Centre for Agri-
23 Environmental Research "Enrico Avanzi" (CiRAA) of the University of Pisa (Italy) in
24 2010/2011 and 2011/2012. The area has a coastal Mediterranean climate with a mean

1 annual precipitation of 844 mm and a mean annual temperature of 15°C. Precipitation is
2 mainly in autumn and early spring, whilst summers are usually dry. In the two
3 experimental years precipitation was exceptionally low (Annex 1), especially in 2011,
4 with negative effects on biomass production of cash crops and weeds.

5 The soil is a loamy typic Xeropsamment (Mazzoncini *et al.*, 2010). The trial was arranged
6 in two fields as part of a long-term experiment named MASCOT (Bàrberi and
7 Mazzoncini, 2006; Mazzoncini *et al.*, 2010), in an area which since 1999 has been
8 managed according to the EU organic farming regulations (EC Reg. 1991/2092 and
9 2007/834). The MASCOT 5-yr crop rotation is maize, durum wheat (*Triticum durum*
10 Desf.), sunflower (*Helianthus annuus* L.), pigeon bean (*Vicia faba* L. var. minor), common
11 wheat (*Triticum aestivum* L.). Green manure, including hairy vetch, is grown in the
12 winter before maize and sunflower.

13 The experimental layout was a split-plot design with three replications, with a sub-plot
14 size of 8 × 8 m. The main plots were characterized by either the presence (GM+) or
15 absence (GM-) of a winter green manure.

16 Hairy vetch cv. Latigo was broadcast seeded at a rate of 100 kg ha⁻¹ on 15 September
17 2010 and 31 October 2011, in the first and second years, respectively. The relatively
18 high seeding rate (100 kg ha⁻¹) was chosen to promote an adequate above ground yield
19 biomass. No fertilization, crop protection or direct weeding measures were applied on
20 hairy vetch until termination, which was done by disc harrowing at the flowering stage
21 (8 April 2011 and 24 April 2012). Averaged across all plots, the biomass produced by
22 the vetch accounted for 1.2 (SD = 0.21) t ha⁻¹ of dry matter in 2011 and 5.3 (SD = 0.99) t
23 ha⁻¹ in 2012, corresponding to a N supply of 27.4 and 194 kg ha⁻¹ respectively. The sub-
24 plots included six different P fertilization (PF) treatments: an unfertilized control (C);

1 rock phosphate (C+a); a green compost amended with ~~phosphorite~~-rock ~~phosphate~~
 2 powder at the beginning of compost production, at a rate of 100 kg t⁻¹ fresh matter (EP);
 3 ~~ae~~ compost obtained from the same raw material as EP but not enriched in P (NEP);
 4 and rock phosphate and a non P-enriched compost (C+b) before maize sowing. All the
 5 composts were produced by the International Center for High Mediterranean Agronomic
 6 Studies (CIHEAM – IAM, Bari, Italy) using the same raw material (clippings from
 7 ~~lawns~~~~lawn~~, ornamental palms and ~~olives~~~~olive~~) in EP, NEP and C+b in both years
 8 (Bustamante *et al.*, 2016; Mihreteab *et al.*, 2016; Ciaccia *et al.*, 2017). Except the control
 9 (C-), all treatments included an amount of compost or rock ~~phosphate~~ powder calculated
 10 to replenish the P deficit of the five-year crop rotation, estimated as 24 kg ha⁻¹ (unpubl.
 11 obs.). Compost treatment and rate of application are reported in Table 1.
 12 Maize (cultivar PR36Y03, Pioneer FAO class 300) was sown on 18 April 2011 and 15
 13 May 2012, respectively, in the first and second years, at a rate of 80,000 seeds ha⁻¹.
 14 Maize phenology was assessed from the early stages ~~to~~until flowering using the BBCH
 15 scale (~~Lancashire~~~~Lancashie~~ *et al.*, 1991). Most measurements were taken during the
 16 critical periods for maize/weed ~~competition, i.e. from~~interaction, ~~between~~ 3- to 14-leaf
 17 stage according to Hall *et al.* (1992). ~~Maize~~ plant height, phenological stage, number of
 18 leaves, mean leaf width, length and area were measured on three plants plot⁻¹ 58 days
 19 after sowing (DAS) (BBCH scale from 31 to 37, mean = 34). In 2011 these data were used
 20 for a preliminary assessment of the potential competitive ability of maize. In 2012, a
 21 higher number of measurements were taken to obtain better estimates: plant height and
 22 phenological phase were assessed 24, 28, 32, 43, 50, 58, 64, 71 and 90 days after maize
 23 sowing, corresponding to mean BBCH values of 15, 16, 18, 31, 32, 35, 52, 60, and 74
 24 respectively. Number of leaves per plant, mean leaf width, length and area, reported as

1 Leaf Area Index (LAI), ~~leaf area unit on ground surface unit~~, were measured 34 and 54
2 DAS. Crop N nutritional status was measured by a chlorophyll meter (SPAD-502, Konica
3 Minolta Holding, Inc.) at 24, 34, 43 and 50 DAS on the fourth and fifth leaves. Total maize
4 biomass was measured only at harvest in 2011, and at 28, 34, 43 DAS in 2012.
5 Weed density by species was measured before post-emergence inter-row cultivation
6 (hoeing), which happened only once in the two years when maize had five unfolded
7 leaves (BBCH 15). Weed density was assessed on three 50 × 50 cm sampling areas plot⁻¹
8 on 20 May 2011 and 8 June 2012, respectively. In 2011, weed biomass was very limited
9 throughout the maize cropping cycle due to drought and thus was not sampled. In 2012,
10 three weed biomass samplings were performed at 28, 34 and 43 DAS, respectively.

11

12 **Statistical analysis**

13 **Maize potential competitive ability**

14 Data from the preliminary study on the level of correlation among maize traits selected
15 as indicators of potential competitive ability (plant height, phenological stage, number of
16 leaves, mean leaf width, length and area) were analysed with a linear model to select
17 independent variables. For 2011 data, we used a split-plot ANOVA upon a Linear Mixed
18 Model with Gaussian distribution. Compost treatments, nested within green manure
19 treatments, were considered as a fixed factor and blocks as a random factor. Tukey's
20 post-hoc test was applied to cases that showed statistical significance. For 2012 data
21 (for which several parameters had repeated measures) time was added in the model as
22 a fixed effect (Faraway, 2005). Where more than one individual plant or plant organ
23 (leaves in the case of SPAD) were sampled, these sampling units were added to the
24 model as the nested random factor. For example, in the error structure of the model

1 concerning SPAD data, measured leaves (the fourth and fifth) were included as nested
2 ~~effect~~ within individual plants, ~~and as~~ the three plants were included ~~as~~ nested ~~effect~~
3 within each sampling ~~area, area~~).

4 When ANOVA pre-assumptions were not met all data were appropriately transformed
5 (Gomez and Gomez, 1984) . Homoscedasticity and normality of residuals were checked
6 with the Bartlett ~~test~~Test (Snedecor and Cochran, 1989) and the Shapiro test (Shapiro
7 and Wilk, 1965), respectively. Linear Mixed Model analyses were performed using the
8 “Lme4” package for R (Bates *et al.*, 2015). When transformation was ineffective in
9 meeting data requirements for ANOVA, a non-parametric Friedman test (Conover, 1980)
10 was used to highlight the factor(s) affecting the dependent variable, and the Conover
11 test (Conover, 1980) was used as post-hoc test.

12 In 2011, due to the severe drought, no weeds emerged in the third block, which was then
13 excluded from the analysis. To increase data accuracy, measurements were repeated
14 three times in each plot: these were considered as pseudo-replications and included in
15 the statistical model as ~~a~~ random effect nested within the block.

16

17 **Weed density and weed community diversity**

18 Using weed density data, the following ~~parameters~~ were calculated: species richness, the
19 Shannon diversity index (HS) (Magurran, 1988), the inverse Simpson index of diversity
20 (invsimp) (Peet, 1974), and Pielou's evenness index (J) (Sheldon, 1969). Weed density
21 was partitioned into functional groups ~~upon in terms of~~ the following response traits: (1)
22 Raunkiær life form for herbaceous species (Raunkiær, 1934), (2) Grime plant strategy
23 (Grime, 1979), (3) two groups based on Ellenberg indicator values for soil fertility (N)
24 (Pignatti *et al.*, 2005); one group composed of species with higher value than the mean

1 of all species present, and the other composed of species with lower values than the
2 mean. To separate the effect of the tested factors on functional groups from their general
3 effect on overall weed density, we calculated the relative density by functional group
4 (sum of the densities of all species belonging to a functional group divided by total weed
5 density, expressed as a percentage). Species richness, diversity indices and relative
6 density of functional groups were considered as independent variables in a split-plot
7 design ANOVA, in a Mixed Effect Model, with a Poisson distribution for weed density and
8 species richness data, Gaussian distribution for HS, invsims and J, and binomial
9 distribution for relative density by functional group. Tukey's post-hoc test was applied
10 where needed.

11

12 **Weed community composition**

13 Weed density data were also used to create a matrix of dissimilarities using the Bray-
14 Curtis dissimilarity index. A permutational multivariate analysis of variance was
15 performed to analyse if and how hairy vetch green manure and composts affected weed
16 community composition. With this analysis the distance matrix among sources of
17 variation can be partitioned and a linear model can be fitted to it. The significance of
18 each explanatory variable was obtained by means of *F*-tests based on sequential sums of
19 squares from permutations of the raw data, restricting permutations within each block
20 in order to take the sampling design into account.

21 The diversity matrix was also used for multivariate analysis through non-metric
22 multidimensional scaling (NMDS), which is considered the most robust unconstrained
23 ordination method in community ecology (Minchin, 1987). For each factor (green
24 manure or P fertilization) with a significant effect on weed species composition (in

Field Code Changed

terms of the results of the permutational multivariate analysis of variance), a scatter plot based on samples was produced. Weed community analyses were carried out using the “vegan” package for R (Oksanen *et al.*, 2009).

Maize/weeds competitive interactions

To understand whether green manure and/or P fertilization gave a competitive advantage to maize or to weeds, a response comparison index (RCI) was computed (Campiglia *et al.*, 2014). This index is based on the relative response index of weed (RRI_w) and crop (RRI_c), calculated as indicated by Williams *et al.* (1998). RRI_w was calculated as:

$$RRI_w = (CBM_w - TBM_w) / (CBM_w + TBM_w)$$

where CBM_w is the weed above ground biomass in the control plot (C⁻ and GM⁻ for the P fertilization and green manure effect, respectively), and TBM_w is the weed aboveground biomass in every other treatment (EP, NEP, C⁺a and C⁺b for the P fertilization effect, and GM⁺ for the green manure effect). RRI_w values < 0 mean that weed biomass is promoted more by a given treatment than the control.

Similarly, RRI_c was computed as:

$$RRI_c = (CBM_c - TBM_c) / (CBM_c + TBM_c)$$

where CBM_c and TBM_c are the crop total above ground biomass in the control and treatment plots, respectively. RRI_c values < 0 mean that crop biomass is more stimulated by a given treatment compared to the control. Finally, the Response Comparison Index was calculated as:

$$RCI = RRI_w - RRI_c$$

Field Code Changed

Field Code Changed

Field Code Changed

1 RCI gives an idea of crop/weed competitive interactions under each treatment. Positive
2 RCI values indicate that the crop is more competitive than weeds, whereas negative RCI
3 values indicate the opposite. Due to the lack of weed biomass data for 2011, RCI was
4 only computed on 2012 data at each sampling date (28, 34 and 43 DAS). When time had
5 a significant effect on the indices, the analyses were performed by sampling date. With
6 respect to the effects of green manure, due to the lower number of values produced by
7 the calculation procedure, time was added as a random factor in a Mixed Effect Model
8 with Gaussian distribution. A Z-test, using the BSDA package for R (Brill, 2005) was run
9 to assess whether RCIs were significantly higher than 0, and whether the RRI were
10 significantly different from 0.
11 Statistical analyses were carried out using R 3.0.3 (R Development Core Team, 2014)
12 with package lme4 (Bates *et al.*, 2015) for mixed models.

13

14 **Results and discussion**

15 **Maize potential competitive ability**

16 In 2011 and 2012 all maize leaf characteristics (number of leaves, mean leaf width,
17 length and area) were significantly ($P < 0.001$) and linearly correlated with leaf area
18 plant^{-1} (see Annex 2), hence only leaf area plant^{-1} results are shown.

19 According to the Friedman rank sum test, the phenological phase of maize in 2011 was
20 significantly ($P < 0.05$, Friedman's chi-squared statistic = 7.69) more advanced in plots
21 where green manure had been applied (median BBCH = 35) compared to plots where
22 GM was not applied (median BBCH ≡ = 33), while in 2012 no significant effect was
23 detected (see Annex 3). With respect to P application, no significant effects on crop
24 phenology were found in either year (data not shown). Leaf area plant^{-1} was significantly

1 increased by green manure application (GM⁺), and was 1.7-fold higher in 2011 at 58
2 DAS, and two- and 1.56-fold higher at 34 and 50 DAS, respectively, in 2012 (Table 2)
3 compared to GM⁻.

4 Regarding the P fertilization levels, only NEP in 2011 showed higher (+25%) values for
5 leaf area plant⁻¹ than the control (C⁻), while there was no significant effect in 2012. In
6 both years, green manure increased early stage plant height: in 2012 maize reached the
7 maximum plant height about 15 days earlier in green manured plots (Annex 3). Table 2
8 shows only plant height data for the period in which the green manure effect was
9 significant, i.e. between 43 (+35%) and 58 DAS (+29%). In 2011 maize plants were 17%
10 taller in NEP than in C⁻, while there was no evident P fertilization effect in 2012. There
11 was no interaction between green manure and P fertilization.

12 Chlorophyll content was affected only by green manure (P = 0.005), which led to an
13 average 28% increase in SPAD unit values compared to GM⁻ (time trends are shown in
14 Annex 3), an effect similar to that found by Radicetti *et al.* (2013). SPAD peak values
15 were observed between 30 and 40 DAS.

16 In 2012, total above ground maize biomass was significantly affected by time and by the
17 time × green manure interaction, therefore data were analyzed separately by date (i.e.,
18 28, 34 and 43 DAS). Green manure increased maize biomass across all sampling
19 ~~dates, dates,~~ In fact the amount of biomass was two-fold, 2.57-fold and 2.46-fold higher
20 than the no green manure treatment at 28, 34 and 43 DAS respectively (Table 2). No
21 significant interaction between green manure and P fertilization was detected.

22 Among all maize parameters considered, the green manure application showed a clear
23 and consistent effect in enhancing plant height and LAI, which are two of the most
24 important traits expressing the potential crop competitive ability against weeds

(Mohammadi, 2007; Zystro *et al.*, 2012). There was a clear positive effect of green manure in the first period of crop development, from 15 to 42 DAS, which is considered to be within the critical period for crop/weed competition (Ferrero *et al.*, 1996). In contrast, P-enriched compost did not have a clearly detectable effect on the ~~maize~~ potential competitive ability of maize.

Weed density, biomass and diversity

Phosphorus fertilization ($P < 0.001$, Chi sq = 65.33, Df = 4) and green manure $\times$ P fertilization interaction ($P < 0.01$, Chi sq = 33.36, Df = 4) significantly affected total weed density in 2011, but not in 2012. In 2011, the differences in total weed density among P fertilization treatments were much lower in GM+ than in GM- plots. In the GM- plots, C+b had 31.5% lower weed density than C-, whereas NEP and C+a had 15.1% and 15.5% higher weed density respectively (Fig. 1). Legume green manures have been reported to increase weed emergence (Blum, 1997; Ciaccia *et al.*, 2015), but not in our study. However, evidence of reduced weed biomass as a result of hairy vetch incorporation in soil was also scarce, and was limited to 28 DAS in 2012 (1.4 vs 4.8 g m⁻² in the GM+ and GM- plots, respectively). At subsequent sampling dates, total weed biomass was similar and rather low (on average 0.6 g m⁻² at 34 DAS and 0.4 g m⁻² at 43 DAS). Neither P fertilization nor the green manure $\times$ P fertilization interaction had a significant effect on weed biomass. The weed biomass reduction observed at 28 DAS in GM+ plots may have been due to an allelopathic effect produced by *V. villosa*, as reported by Bittencourt *et al.* (2013).

The number of species (S) and the Shannon index (H') were not affected by green manure or P fertilization in either year (average S: 4.6 in 2011 and 7.0 in 2012; average

Formatted: Font: Cambria

Formatted: Font: Italic

1 H': 1.85 in 2011 and 1.57 in 2012). On the other hand, the Inverse Simpson index was
2 significantly higher ($P < 0.05$) in plots where green manure was incorporated in-to the
3 soil (1.30) than in those without green manure (1.25). Comparing the results of Shannon
4 and Inverse Simpson indices, as suggested by Morris *et al.* (2014), reveals that the weed
5 community diversity was mainly driven by common species in the first year and by rare
6 species in the second year. Results for N and P dynamics (especially in 2012) showed
7 higher values in GM+ at early growth stages (Ciaccia, 2014). Despite these significant
8 differences observed, which are considered among the main levers for change in weed
9 diversity (Hawes *et al.*, 2010 for N; Wassen *et al.*, 2005 for P), the practices tested in our
10 study did not deplete weed community diversity. In fact, the only significant effect
11 exerted by green manure (in only one year) was to increase the diversity of common
12 weed species, shifting the weed community to a more balanced composition (i.e., with a
13 high number of evenly distributed ~~species;species~~ Bàrberi, (2002). A more diverse
14 weed ~~weed~~-flora is less competitive with the cash crop, ~~and~~ can minimize the risks of
15 predominance of a few competitive species, ~~which that~~ occupy specific ecological niches,
16 ~~by~~ competing with the cash crops for the same resources (Poffenbarger *et al.*, 2015;
17 Storkey ~~and~~ Neve, 2018).

Formatted: Font: Italic

19 **Weed community composition**

20 According to the results of the permutational multivariate analysis of variance (Table 3),
21 green manure was the only factor that significantly affected weed community
22 composition at an early stage in both years ($P < 0.01$), the magnitude of the effect was
23 higher in 2012 than in 2011. Not surprisingly, P application had little effect on weed

community composition at that stage, since P availability did not differ among the several P fertilization treatments until stem elongation (Ciaccia, 2014).

NMDS highlighted a clear green manure effect on weed assemblages in both years (Figures 2a and 2b). ~~2011 (Figure 2a), despite a strong block effect.~~ Species affinity with green manure application was consistent between years (compare Figures 2a and 2b).

In particular, green manure selected for annual nitrophilous dicotyledonous species (~~Annex 4 are reported~~ the Ellenberg N values for each species sampled are reported in Annex 4) such as *Anagallis arvensis* L. in 2011 and *Stellaria media* (L.) Vill. ~~in 2012~~, whereas ~~it selected against the green manure did not favour~~ annual grasses such as *Lolium multiflorum* Lam. and perennial species such as *Cyperus esculentus* L.

~~In 2012 the block effect was lower, thus the effect of the green manure was more noticeable than in 2011.~~ Competition for resources exerted by the presence of hairy vetch likely reduced the possibility of accumulating photosynthates in underground storage organs in species (e.g. perennials) for which this represents a key survival strategy.

Formatted: Font: Not Bold

Weed community functional analysis

~~In 2011~~ accordance with the Raunkiaer life form methodology, we classified the observed species into the three categories of therophytes (i.e., mostly annual plants that reproduce by seeds), geophytes (i.e., perennial plants that resprout from underground vegetative organs), and hemicryptophytes (i.e., plants that resprout from buds placed at a soil level).

~~In 2011, the relative density of the Raunkiaer life forms—considered~~ was never significantly affected by the experimental factors. In 2012, due to a very high incidence of therophytes ~~species incidence~~ (in 75.6% of the samples, the only species present were

therophytes), no such analysis was performed. Regarding Grime's plant strategy groups, the relative density of ruderals (R) was 100% and 42% higher in green manured plots in 2011 and 2012 respectively than in plots where green manure was not applied. Similarly, competitive-ruderals (CRs) were favored by hairy vetch incorporation in 2012 (relative density: 39.8%) (Table 4).

Species with a high affinity for rich soils (i.e. Ellenberg indicator values for soil fertility > 5, see Pignatti *et al.*, 2005 and list in Annex 4) were not significantly affected by green manure or compost type in either years (data not shown), despite the higher levels of soil N found after green manure incorporation (Ciaccia, 2014).

Our study ~~suggests~~~~would seem to highlight~~ that P application, in the form of P-enriched compost, ~~does~~~~did~~ not affect the magnitude of weed emergence, unlike Blackshaw and Molnar (2009) ~~who~~~~who~~ found a significant effect ~~offer~~ P ~~application~~~~applications~~. However, the ~~se~~~~latter~~ authors ~~did not use~~~~used~~ P ~~sources different by~~ enriched compost ~~as P source~~.

On the other hand ruderal and competitive-ruderal species seem to find more favorable conditions after green manure application. Farmers should thus monitor the abundance of such species in subsequent spring-sown crops and use appropriate control strategies when necessary. This ~~would be~~~~is~~ particularly ~~important~~~~true~~ in case of high presence of weed species ~~emerging~~~~which emerge~~ at the same time as maize, ~~thus potentially more competitive~~~~grow together, compete for water and soil nutrients and attain similar heights at maturity~~ (e.g. *Xanthium strumarium* L., *Datura stramonium* L., *Echinochloa crus-galli* L., *A. retroflexus*, *Sorghum halepense* L., *Chenopodium album* L.). ~~This could lead to~~, ~~thus leading to~~ a potentially high yield ~~reduction~~~~depletion~~ (Gołębiewska and Kieloch, 2016; Yousefi *et al.*, 2015).

Formatted: Font: Italic

1

2 **Maize/weed competition**

3 When considering the effect of green manure on weed and crop competition, neither the
4 RRI or RCI indices showed any significant interaction between green manure and P
5 fertilization (Chi-square: 1.446 and 2.515 for RRI_c in 2011 and 2012; 1.102 for RRI_w in
6 2012, and 1.562 for RCI in 2012). In both years, the results of the Z-test on RRI clearly
7 indicated a ~~significant~~ competitive advantage for maize when grown after green manure
8 ($RRI_c < 0$), while weeds (in 2012) were not favored by green manure application (RRI_w
9 not significantly higher or lower than 0), as reported in Table 5. ~~As a consequence,~~
10 ~~the~~The RCI for the green manure factor ~~thus~~ showed that crop/weed competitive
11 relationships favored the crop to the detriment of weeds when green manure was
12 applied.

13 This result has very important practical implications ~~and~~ ~~This~~ is consistent with
14 Liebman and Davis (2000), who found that the competitive ability of the cash crop was
15 enhanced by the application of leguminous green manure. Although we did not measure
16 the N content in weeds, we can hypothesize that the high soil N availability and N uptake
17 by maize during the critical period for crop/weed competition (Ciaccia *et al.*, 2017),
18 following green manure incorporation, played a key role in shifting the competitive
19 balance towards the crop. As shown in Table 2, maize traits related to competitive
20 ability (plant height, leaf area) had higher values ~~in~~following green manured crops, an
21 effect most likely driven by higher N availability.

22 The RRI and RCI indices that were calculated to study the effect of the P fertilization
23 factor on crop/weed competition (Table 6) also indicated a competitive advantage for
24 the cash crop (negative RRI_c and positive RRI_w) when P was applied. However, the way P

1 was applied did not significantly affect the results (Chi-square: 0.655 and 3.876 for RRI_c
2 in 2011 and 2012; 7.211, 8.039 and 1.442 for RRI_w at 28, 34 and 43 DAS respectively in
3 2012; 2.535, 9.414 and 0.447 for RCI at 28, 34 and 43 DAS respectively in 2012). In
4 contrast, RRI_w and RCI calculated at 34 DAS in 2012 revealed that ~~weeds~~~~the weed~~ were
5 more competitive when P was applied together with green manure, and ~~that~~~~by~~ maize
6 ~~was more competitive~~ when P was applied alone (Table 6). Although this effect was
7 found just in one of the three sampling dates, ~~when~~ N and P are more available for
8 weeds they may stimulate weed growth even in a quite N-demanding crop like maize (in
9 accordance with Di Tomaso, ~~(1995)~~ and Davis and Liebman, ~~2001~~, ~~(2001)~~). However,
10 in our study the ~~overall~~~~general~~ effect of green manure and P fertilization was to increase
11 the competitive ability of maize against weeds.

12

13 Conclusions and recommendations

14 The data presented in this paper confirm that the complexity of interactions between
15 ~~two of the most important agroecosystem elements~~ soil fertility, ~~crop~~ and weeds can
16 be ~~steered towards a crop benefit~~~~overcome~~ by implementing agronomic techniques that
17 increase soil fertility whilst keeping weeds under control.-

18 Combining high N₂-fixing legume green manures with P-enriched amendments can
19 promote nutrient cycling in organic stockless cropping systems, where the absence of
20 farmyard manure may lead to a shortage of ~~soil~~ N and P in the ~~soil in the~~ long term.
21 Nevertheless, in our conditions ~~there was no immediate~~~~prompt~~ effect of P-enriched
22 compost on early maize growth, ~~was not observed~~. Likewise, the application of green
23 manure and/or P enriched compost did not increase early stage weed infestation in
24 maize. We argue that the dry conditions might have indeed slowed down the

Formatted: Standard

1 mineralization rate of the green manure and of the composts, leading to no
2 ~~immediate prompt~~ availability of N_i and, especially, P_i for ~~either both~~ maize ~~or and~~ weed
3 plants. Further research under more humid ~~spring~~ conditions ~~is in spring are~~ thus
4 required to unravel the ~~actual~~ potential of these two nutrient sources ~~of in~~ suppressing
5 ~~weeds or favoring weed populations~~ in maize. Soil incorporation of a hairy vetch green
6 manure clearly enhanced the expression of ~~the~~ competitive traits of maize (i.e., plant
7 height and LAI) and, overall, the competitive ability of maize against weeds. On the other
8 hand, green manure shifted weed community composition towards a higher relative
9 abundance of ruderal and competitive-ruderal species. These dynamics should be
10 monitored if these species become dominant, in which case dedicated management
11 tactics would need to be implemented. We found that hairy vetch green manure reduced
12 the development of ~~weed groups of~~ species ~~groups such as creepers and like creeping~~
13 perennials (e.g. *C. esculentum*), ~~which are often that can be considered~~ detrimental to
14 maize production. Overall, the weed community shifts induced by green manure seem to
15 lead to a less aggressive weed community for subsequent spring-summer crops. However
16 this needs to be confirmed in a longer-term perspective, in particular considering the very
17 dry conditions of the two years studied during our ~~tests, test.~~

18

19 **Acknowledgements**

20 This study was part of the COMPARABIMUS project, funded by the Italian Ministry of
21 Agricultural, Food and Forestry Policies (MiPAAF). The authors wish to thank Giacomo
22 Nardi and the CIRAA staff for their technical support.

1 Reference

- 2 Anugroho, F., Kitou, M., Nagumo, F., Kinjo, K., ~~and~~ Tokashiki, Y. 2009. Growth, nitrogen
3 fixation, and nutrient uptake of hairy vetch as a cover crop in a subtropical
4 region. ~~Weed Biology~~ ~~biology~~ and ~~Mangement~~ ~~management~~ 9(1): 63-71.
- 5 Bàrberi, P. 2002. Weed management in organic agriculture: are we addressing the right
6 issues? Weed Research 42(3): 177-193.
- 7 Bàrberi, P., and Mazzoncini, M. 2001. Changes in weed community composition as
8 influenced by cover crop and management system in continuous corn. Weed
9 Science 49(4): 491-499.
- 10 Bàrberi, P., and Mazzoncini, M. 2006. The MASCOT (Mediterranean Arable Systems
11 Comparison Trial) long-term experiment (Pisa, Italy). Long-term Field
12 experiments in Organic Farming. ~~ISO FAR~~ ~~Isofar~~. Sci. Series (1): 1-15.
- 13 Bates, D., Maechler, M., Bolker, B. and Walker, S. 2015. Fitting Linear Mixed-Effects
14 Models Using lme4. Journal of Statistical Software 67(1): 1-48.
- 15 Bittencourt, H. V. H., Lovato, P. E., Comin, J. J., Lana, M. A., Altieri, M. A., Costa, M. D., and
16 Gomes, J. C. 2013. Effect of winter cover crop biomass on summer weed
17 emergence and biomass production. Journal of Plant Protection Research 53(3):
18 248-252.
- 19 Blackshaw, R. E. and Brandt R. N. 2008. Nitrogen fertilizer rate effects on weed
20 competitiveness is species dependent. Weed Science 56(5): 743-747.
- 21 Blackshaw, R. E., and Molnar, L. J. 2009. Phosphorus fertilizer application method affects
22 weed growth and competition with wheat. Weed Science 57(3): 311-318.

- 1 Blackshaw, R. E., and Brandt, R. N. (2009). Phosphorus fertilizer effects on the
2 competition between wheat and several weed species. *Weed Biology and*
3 *Management* 9(1): 46-53.
- 4 Blackshaw, R. E., Brandt, R. N., Henry Janzen, H., and Entz, T. 2004. Weed species
5 response to phosphorus fertilization. *Weed Science*, 52(3): 406-412.
- 6 Blum, U., King, L. D., Gerig, T. M., Lehman, M. E., and Worsham, A. D. 1997. Effects of
7 clover and small grain cover crops and tillage techniques on seedling emergence
8 of some dicotyledonous weed species. *American Journal of Alternative*
9 *Agriculture* 12(4): 146-161.
- 10 Brandsæter, L. O., Heggen, H., Riley, H., Stubhaug, E., and Henriksen, T. M. 2008. Winter
11 survival, biomass accumulation and N mineralization of winter annual and
12 biennial legumes sown at various times of year in Northern Temperate Regions.
13 *European Journal of Agronomy* 28(3): 437-448.
- 14 Brill, R. V. 2005. Basic Statistics and Data Analysis. *Technometrics* 47(1). CA USA.
- 15 Bustamante, M. A., Ceglie, F. G., Aly, A., Mihreteab, H. T., Ciaccia, C., and Tittarelli F. 2016.
16 Phosphorus availability from rock phosphate: Combined effect of green waste
17 composting and sulfur addition. *Journal of Environmental Management* 182: 557-
18 563.
- 19 Campiglia, E., Radicetti, E., Brunetti, P., and Mancinelli, R. 2014. Do cover crop species
20 and residue management play a leading role in pepper productivity? *Scientia*
21 *Horticulturae*, 166: 97-104.
- 22 Ciaccia, C. 2014. Effetto combinato del sovescio e del compost sulla dinamica di azoto e
23 fosforo in sistemi culturali arativi biologici senza zootecnia (stockless). PhD

Formatted: Italian (Italy)

Formatted: Font: (Default) Cambria, Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

1 Thesis, Retrieved from ETD database number: 07232014-143443, University of
2 Pisa.

3 Ciaccia, C., Ceglie, F., Tittarelli, F., Antichi, D., Carlesi, S., Testani, E., and Canali, S. 2017.
4 Green manure and compost effects on NP dynamics in Mediterranean organic
5 stockless systems. *Journal of Soil Science and Plant Nutrition*, 17(3): 751-769.

6 Ciaccia, C., Montemurro, F., Campanelli, G., Diacono, M., Fiore, A., ~~and~~ Canali, S. 2015.
7 Legume cover crop management and organic amendments application: Effects on
8 organic zucchini performance and weed competition. *Scientia Horticulturae*, 185:
9 48-58.

10 Conover, W. J. 1980. *Practical nonparametric statistics*,. New York, USA.

11 Davis, S.A. and Liebman, M., 2001. Nitrogen source influence wild mustard growth and
12 competitive effect on sweet corn. *Weed Science* 49 (4): 558-566.

13 Di Tomaso, J.M., 1995. Approaches for improving crop competitiveness through the
14 manipulation of fertilization strategies. *Weed Science* 43 (3): 491-497.

15 Faraway, J. J., 2005. *Extending the linear model with R: generalized linear, mixed effects*
16 *and nonparametric regression models*. Boca Raton, Florida: CRC press.

17 Ferrero, A., Scanzio, M., and Acutis, M. 1996. Critical period of weed interference in
18 maize. *Proceedings of the Second International Weed Control Congress*.
19 Copenhagen, Denmark: Department of Weed Control and Pesticide Ecology: 171-
20 176.

21 Gomez, K. A., and Gomez A. A. 1984. *Statistical procedures for agricultural research*. New
22 York, USA: John Wiley & Sons.

23 Grime, J. P. 1979. *Plant strategies and vegetation processes*. Chichester, New York,
24 Brisbane, Toronto: John Wiley & Sons.

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

- 1 Grant, C. A., Flaten, D. N., Tomasiewicz, D. J., and Sheppard, S. C. ~~2001.(2001).~~ The
2 importance ~~—~~of early season phosphorus nutrition. Canadian Journal of Plant
3 Science 81 (2): 211-224.
- 4 Hall, M. R., Swanton, C. J., and Anderson, G. W. ~~1992.(1992).~~ The critical period of weed
5 control i——n grain corn (*Zea mays*). Weed ~~Science~~*science* 40_(3): 441-447.
- 6 Hartwig, N. L., and Ammon, H. U. ~~2002.(2002).~~ Cover crops and living mulches. Weed
7 Science 50_(6): 688-699.
- 8 Hawes, C., Squire, G. R., Hallett, P. D., Watson, C. A., and Young, M. 2010. Arable plant
9 communities as indicators of farming practice. Agriculture, Ecosystems &
10 Environment 138_(1): 17-26.
- 11 Hoveland, C. S., Buchanan, G. A., ~~and~~& Harris, M. C. 1976. Response of weeds to soil
12 phosphorus and potassium. Weed Science 24_(2): 194-201.
- 13 Karasawa, T., and Takahashi, S. 2015. Introduction of various cover crop species to
14 improve soil biological P parameters and P uptake of the following crops.
15 Nutrient Cycling in Agroecosystems 103_(1): 15-28.
- 16 Lancashire, P. D., Bleiholder, H., Boom, T. V. D., Langelüddeke, P., Stauss, R., Weber, E.,
17 and Witzemberger, A. 1991. A uniform decimal code for growth stages of crops
18 and weeds. Annals of Applied Biology 119_(3): 561-601.
- 19 Lehoczy, É., Gólya, G., Tamás, J., and Németh, T. 2015. Biodiversity and Biomass
20 Production of Weeds in a Long-Term Fertilization Experiment. Communications
21 in Soil Science and Plant Analysis 46. (~~Suppl. 1~~): 390-398.
- 22 Liebman, M., and Davis A. S. 2000. Integration of soil, crop and weed management in
23 low-external-input farming systems. Weed Research, 40_(1): 27-48.

Formatted: Indent: Left: 0", Hanging: 0.49"

Formatted: Font: Italic

1 Magurran, A.E. 1988. Ecological Diversity and its Measurement. Princeton University
2 Press, Princeton.

3 Mazzoncini, M., Canali, S., Giovannetti, M., Castagnoli, M., Tittarelli, F., Antichi, D.,
4 Nannelli, R., Cristani, C., and Bàrberi, P. 2010. Comparison of organic and
5 conventional stockless arable systems: A multidisciplinary approach to soil
6 quality evaluation. *Applied Soil Ecology* 44 (2): 124-132.

7 Mihreteab, H. H. T., Ceglie, F. G., Aly, A., and Tittarelli, F. 2016 Rock phosphate enriched
8 compost as a growth media component for organic tomato (*Solanum*
9 *lycopersicum* L.) seedlings production. *Biological Agriculture and Horticulture*,
10 Vol. 32 (1): 7-20.

11 Minchin, P. R. 1987. An evaluation of the relative robustness of techniques for ecological
12 ordination. In: Theory and Models in ~~Vegetation Science:vegetation~~
13 ~~science~~: 89-107. Springer Netherlands.

14 Mohammadi, G. R. 2007. Growth parameters enhancing the competitive ability of corn
15 (*Zea mays* L.) against weeds. *Weed Biology and Management*, 7 (4): 232-236.

16 Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., Meiners, T.,
17 Muller, C., Obermaier, E., Prati, D., and Rillig, M. C. 2014. Choosing and using
18 diversity indices: insights for ecological applications from the German
19 Biodiversity Exploratories. *Ecology and Evolution*, 4 (18): 3514-3524.

20 Njeru, E. M., Avio, L., Sbrana, C., Turrini, A., Bocci, G., Bàrberi, P., and Giovannetti, M.
21 2014. First evidence for a major cover crop effect on arbuscular mycorrhizal
22 fungi and organic maize growth. *Agronomy for Sustainable Development* 34 (4):
23 841-848.

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

1 Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H.,
2 and Wagner, H. 2009. Vegan: community ecology package. R package version
3 1.15-2. R Foundation for Statistical Computing, Vienna, Austria.

4 Owla, M. L., Nepalia, V., Chouhan, G. S., and Singh, D. 2015. Effect of fertility levels,
5 nutrient sources and weed control on weed dynamics and yield of quality protein
6 maize (*Zea mays*) and relative nitrogen and phosphorus uptake. Indian Journal of
7 Agronomy, 60,(2): 267-272.

8 Peet, R. K. 1974. The Measurement of Species Diversity. Annual Review of Ecology and
9 Systematics 5: 285-307.

10 Pignatti, S., Menegoni, P., and Pietrosanti, S. ~~2005.(2005),~~ Bioindicator values of vascular
11 plants of the Flora of Italy. Braun-Blanquetia 39: 3-95.

12 R Development Core Team 2014. R: A language and environment for statistical
13 computing. R Foundation for Statistical Computing, Vienna, Austria. URL
14 <http://www.R-project.org/>.

15 Radicetti, E., Mancinelli, R., and Campiglia, E. 2013. Influence of winter cover crop
16 residue management on weeds and yield in pepper (*Capsicum annuum* L.) in a
17 Mediterranean environment. Crop Protection 52,(10): 64-71.

18 Rajcan, I., and Swanton, C. J. 2001. Understanding maize–weed competition: resource
19 competition, light quality and the whole plant. Field Crops Research 71,(2): 139-
20 150.

21 Raunkiaer, C. ~~1934.(1934)~~. The life forms of plants and statistical plant geography; being
22 the collected papers of C. Raunkiaer. Clarendon Press, Oxford.

23 Reddy, K. N., and Koger, C. H. 2004. Live and killed hairy vetch cover crop effects on
24 weeds and yield in glyphosate-resistant corn. Weed Technology 18,(3): 835-840.

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

1 Rotchés-Ribalta, R., Armengot, L., Mäder, P., Mayer, J., and Sans, F. X. ~~2017.(2017).~~ Long-
2 term management affects the community composition of arable soil seedbanks.
3 Weed Science, ~~65~~₍₁₎: 73-82.

4 Shapiro, S. ~~S.S.~~ and ~~M. B.~~ Wilk, ~~M. B.~~ 1965. An analysis of variance test for normality
5 (complete samples). Biometrika ~~52 (3 and 4)~~_: 591-611.

6 Sheldon, A. L. 1969. Equitability indices: dependence on the species count. Ecology ~~50~~
7 (3): 466-467.

8 Snedecor, G. W. and Cochran, W. G. 1989. Statistical Methods, Eighth Edition, Iowa State
9 University Press.

10 Storkey, J., ~~and~~& Neve, P. 2018. What good is weed diversity?. Weed Research, 58 (4):
11 239- 243.

12 Sweeney, A. E., Renner, K. A., Laboski, C., and Davis, A. 2008. Effect of fertilizer nitrogen
13 on weed emergence and growth. Weed Science, 56₍₅₎: 714-721.

14 Tang, L., Cheng, C., Wan, K., Li, R., Wang, D., Tao, Y., Pan, J., Xie, J., and Chen, F.
15 ~~2014.(2014).~~ Impact of fertilizing pattern on the biodiversity of a weed
16 community and wheat growth. Plos ~~One,one~~_{, 9}₍₁₎: e84370.

17 Thomas, P. E. L., and Allison, J. C. S. 1975. Competition between maize and *Rottboellia*
18 *exaltata*. The Journal of Agricultural Science, 84₍₂₎: 305-312.

19 Tollenaar, M., Nissanka, S. P., Aguilera, A., Weise, S. F. and Swanton, C. J. 1994. Effect of
20 weed interference and soil nitrogen on four maize hybrids. Agronomy Journal, 86
21 (4): 596-601.

22 Wassen, M. J., Venterink, H. O., Lapshina, E. D., and Tanneberger, F. 2005. Endangered
23 plants persist under phosphorus limitation. Nature 437₍₇₀₅₈₎: 547-550.

Formatted: Font: Not Italic

Formatted: Font color: Auto

Formatted: Font color: Auto

- 1 Williams, M. M., Mortensen, D. A., and Doran, J. W. 1998. Assessment of weed and crop
- 2 fitness in cover crop residues for integrated weed management. *Weed Science* 46
- 3 (5): 595-603.
- 4 Zystro, J. P., de Leon, N., and Tracy, W. F. 2012. Analysis of traits related to weed
- 5 competitiveness in sweet corn (*Zea mays* L.). *Sustainability* 4 (4): 543-560.

Formatted: Font: Italic

1 Figures captions:

2 Figure1: Total weed density as compost x green manure (GM) interaction; error bars
3 represent the standard error (SE). GM presence is reported in grey, GM absence
4 in black. (C-, untreated –no compost, no rock phosphate-; C+a, rock phosphate
5 only; C+b, Compost + rock phosphate; EP, P enriched compost; NEP, compost
6 only; composition and rate of application are reported in Table1).

7 Figure 2a: Site ordination (NMDS) based on floristic similarities of 20 plots (k=2, non-
8 metric fit: $R^2=0.953$, stress=0.215). GM, Green manure. Species names are
9 reported in Annex 4.

10 Figure 2b. Site ordination (NMDS) based on floristic similarities of 30 plots (k=2, non-
11 metric fit: $R^2=0.939$, Stress: 0.246).GM, Green manure. Species names are
12 reported in Annex 4.

13 December 2018

To whom it may concern

This is to declare that I have edited and proofread the English of the following paper:

Green manure and phosphorus fertilization affect weed community composition and crop/weed competition in organic maize

On behalf of:

Antichi et al

My revision did not include the Bibliography. Subsequent to my revision, the authors may have made other changes or chosen not to implement some of the changes that I suggested. The correctness of the technical terms is also the responsibility of the authors.

The final version of the manuscript, as sent to the authors on **13 December 2018**, will be kept in our archives.

I have 30 years of experience of editing the English of scientific papers. I am also the author of *English for Writing Research Papers* published by Springer.

Please do not hesitate to contact me for any further information you may require:

adrian.wallwork@gmail.com

Best regards

A handwritten signature in blue ink, appearing to read 'Adrian Wallwork', is positioned above the printed name.

Adrian Wallwork

- 1
- Tables
- 2
- Table 1: P fertilization treatment application rates expressed as total materials and total P₂O₅.

		Compost (kg ha ⁻¹)	P- enriched compost (kg ha ⁻¹)	Rock phosphate (kg ha ⁻¹)	Total P ₂ O ₅ (kg ha ⁻¹)
Treatment	Compost factor				
C-	Untreated (No compost, no rock phosphate)	0	0	0	0
C+a	Rock phosphate	0	0	204	24
C+b	Compost + rock phosphate	3,619	0	158	24
EP	P-enriched compost	0	3,478	0	24
NEP	Not P-enriched compost	16,250	0	0	24

3

1 Table 2: Maize leaf area, height and biomass in 2011 and 2012 in the different green manure and phosphorus fertilization
2 (PF) treatments.

	Maize leaf area (cm ²)			Maize height (cm)					Maize biomass (g)			
	2011	2012		2011	2012				2011	2012		
	58 DAS	34 DAS	50 DAS	58 DAS	32 DAS	43 DAS	50 DAS	58 DAS	149 DAS	28 DAS	34 DAS	43 DAS
Green manure	***	***	*	***	NS	***	**	NS	***	***	***	***
F value	129.24	16.75	11.62	118.63	0.78	89.60	38.64	1.01	17.38	38.37	123.07	74.33
GM ⁺	3174.45 a	1215.22 a	2228.77 a	139.55 a	65.93	109.17a	123.97 a	125.90	1610.51 a	4.08 a	12.86 a	38.78 a
GM ⁻	1870.88 b	598.60 b	1421.66 b	88.13 b	55.73	80.97b	95.80 b	115.41	1221.60 b	2.01 b	5.02 b	15.92 b
PF treatment	*	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS
F value	2.84	0.71	0.82	6.07	2.05	1.59	0.88	0.97	1.77	0.75	0.20	0.24
C ⁻	2294.56 b	881.81	2046.76	108.79 b	61.83	98.33	114.75	125.86	1231.52	2.94	8.95	30.04
C ^{+a}	2594.49 ab	945.31	1873.73	116.67 ab	58.33	94.75	111.42	122.25	1459.72	2.91	9.09	24.51
C ^{+b}	2412.26 ab	964.38	1581.31	106.27 ab	57.67	89.67	105.67	115.42	1351.49	3.36	9.05	23.49
EP	2453.83 ab	847.58	1743.95	109.54 ab	64.42	92.58	106.58	120.87	1462.03	3.11	8.37	27.12
NEP	2858.18 a	895.48	1880.33	127.25 a	61.92	100.00	111.00	118.89	1575.50	2.91	9.23	31.63
Green manure X PF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F value	0.62	1.08	1.61	2.19	0.32	1.66	0.31	1.59	0.31	0.33	0.56	0.50

3 DAS = days after sowing. GM⁺ = application of green manure, GM⁻ = plots without green manure, C⁻ = untreated, C^{+a} = rock phosphate, C^{+b} = compost
4 + rock phosphate, EP = P-enriched compost, NEP = not P-enriched compost. F values and significance based on Generalized Linear Model ANOVA are
5 reported. Degrees of freedom for green manure, PF and green manure x PF interaction are 1, 4 and 4 respectively. *, **, *** = significant at P < 0.05, 0.01
6 and 0.001 respectively; NS = not significant

1 Table 3: Sum of squares, partial r-squared and its significance for weed flora composition as
 2 explained by Green manure, phosphorus fertilisation (PF) and Green manure x PF interaction in
 3 2011 and 2012.

Years	2011		2012	
	SS	r ²	SS	r ²
Green manure	0.213	0.084**	0.638	0.196***
PF	0.391	0.154	0.351	0.108
Green manure x PF	0.277	0.109	0.224	0.069
Total	2.524		2.727	

4
 5 Values are based on permutational multivariate analysis of variance (999 permutations). **, ***Significant
 6 at P < 0.01 and 0.001 respectively

1 Table 4. Effect of green manure, phosphorus fertilisation (PF) and green manure x PF interaction
2 on the relative density of Ruderals and Competitive-Ruderals weeds.

		Ruderals (%)		Competitive-Ruderals (%)	
		2011	2012	2011	2012
Green manure		**	*	NS	*
	Chi-square	9.988	3.953	0.004	4.930
GM ⁺		17.0 a	48.4 a	0.1	33.6 a
GM ⁻		8.5 b	34.2 b	0.1	24.1 b
PF		NS	NS	NS	NS
	Chi-square	5.139	3.359	4.297	3.141
C ⁻		7.0	42.6	7.3	31.0
C ^{+a}		12.4	52.1	12.4	28.3
C ^{+b}		16.0	36.7	4.1	28.3
EP		13.2	36.8	10.4	30.3
NEP		14.9	38.5	12.3	26.4
Green manure x PF		NS	NS	NS	NS
	Chi-square	2.697	1.476	1.594	2.075

3 GM⁺ = application of green manure, GM⁻ = plots without green manure, C⁻ = untreated, C^{+a} = rock
4 phosphate, C^{+b} = compost + rock phosphate, EP = P-enriched compost, NEP = not P-enriched compost.
5 ANOVA results based on Generalized Linear Model (binomial distribution, log-link function) are reported.
6 Degrees of freedom for green manure, PF and green manure x PF interaction are 1, 4 and 4 respectively. *,
7 **Significant at P < 0.05 and 0.01 respectively; NS = not significant
8

Table 5: Effect of green manure on Relative Response Index of crop (RRlc) and weeds (RRlw) and Response Comparison Index (RCI) in 2011 and 2012

	RRlc		RRlw ¹	RCI
	2011	2012	2012	2012#
Mean	-0.146	-0.387	0.045	0.431
Z-test	-5.002	-15.882	0.412	4.040
P (Y>0)	>0.999	>0.999	0.340	<0.001
P (Y<0)	<0.001	<0.001	0.660	>0.999

¹In 2011 due to adverse climatic conditions weed biomass data were not collected. Error DF= 4.

P (Y>0) = P value, given by the Z test, the null hypothesis is "Y value is higher than zero"; P (Y<0) = P value, given by the Z test, the null hypothesis is "Y value is lower than zero". Significant P values (P<0.05) are shown in bold type face.

1 Table 6: Effect of Phosphorus fertilization on Relative Response Index of crop (RRIC) and weeds (RRIw) and Response Comparison Index (RCI) in
2 2011 and 2012

	RRIC		RRIw (2012)#			RCI (2012)#		
PF effect ¹	2011	2012	28 DAS	34 DAS	43 DAS	28 DAS	34 DAS	43 DAS
Mean	-0.095	-0.251	0.038	0.045	0.325	0.007	0.580	0.959
Z-test	-3.386	-4.371	0.862	1.924	2.191	0.074	2.685	3.507
P (Y>0)	0.999	0.999	0.194	0.973	0.014	0.471	0.996	0.999
P (Y<0)	<0.001	<0.001	0.806	0.027	0.986	0.529	0.004	<0.001
GM x PF interaction²								
Significance	NS	NS	NS	*	NS	NS	*	(*)
Chi-square	2.139	1.086	1.814	9.828	3.033	0.045	6.331	3.131
GM ⁺	-0.030	-0.092	0.107	-0.154	-0.041	-0.015	-0.020	0.208
GM ⁻	-0.146	-0.409	-0.032	0.752	0.690	0.029	1.181	1.457

3
4 P (Y>0) = P value, given by the Z test, the null hypothesis is “Y value is higher than zero”; P (Y<0) = P value, given by the Z test, the null hypothesis is “Y value is
5 lower than zero”. Significant P values (P<0.05) are shown in bold type face.

6 PF = phosphorus fertilization (mean of all treatments but the control); GM = green manure application. (*),*Significant at P<0.10 and 0.05 respectively; ¹Error
7 DF=3; ²Error DF=1.

Figure1: Total weed density, collected the 8th June 2012, as compost x green manure (GM) interaction; error bars represent the standard error (SE). GM presence is reported in grey, GM absence in black. (C-, untreated –no compost, no rock phosphate-; C+a, rock phosphate only; C+b, Compost + rock phosphate; EP, P enriched compost; NEP, compost only; composition and rate of application are reported in Table1).

Species density 20.05.2011

Species density 08.06.2012

Figure 1 and 2 with captions

Figure1: Total weed density, collected the 8th June 2012, as compost x green manure (GM) interaction; error bars represent the standard error (SE). GM presence is reported in grey, GM absence in black. (C-, untreated –no compost, no rock phosphate-; C+a, rock phosphate only; C+b, Compost + rock phosphate; EP, P enriched compost; NEP, compost only; composition and rate of application are reported in Table1).

Species density 20.05.2011

Figure 2a: Site ordination (NMDS) based on floristic similarities of 20 plots ($k=2$, non-metric fit: $R^2=0.953$, stress=0.215). GM, Green manure. Species names are reported in Annex 4.

Species density 08.06.2012

Figure 2b. Site ordination (NMDS) based on floristic similarities of 30 plots ($k=2$, non-metric fit: $R^2=0.939$, Stress: 0.2460867). GM, Green manure. Species names are reported in Annex 4.

(a)

(b)

Annex1: Precipitation and temperature (max e min) reported each ten days, during the maize growing period in 2011(a) and 2012(b), measured in San Piero a Grado (PI).

Annex 2: Linear correlation between: number of leaf (n.leaf), Leaf area index (LAI), length of leaf (Length), average leaf width (Av.Width). Data reported where collected in 2011 (58 days after sowing) and in 2012 (34 and 45 days after sowing)). In the upper plot is reported the R^2 , in the lower part the scatter plot. For R^2 higher than 0.372 $P < 0.01$.

Annex 3: GM effects on time (day after sowing), collected in 2012, clock wise starting from upper left maize height (cm), phenological phase according to the BBCH scale, SPAD values on the 5th leaf, SPAD values on the 4th leaf. The values for GM+ are represented in blue green, GM- in red.

EPPO Code	Latin Name	Raunkiaer ¹	Grime strategy ²	Elleberg N values ³
AGRRE	<i>Agropyron repens (L.) Beauv.</i>	G	CS	8
AMARE	<i>Amaranthus retroflexus L.</i>	T	CR	9
AMIMA	<i>Ammi majus L.</i>	T	CR	2
ANAGAR	<i>Anagallis arvensis L.</i>	T	R	6
BEAVU	<i>Beta vulgaris L.</i>	H	CS	5
CAGSE	<i>Calystegia sepium (L.) R. Br.</i>	H	CR	9
CAPBP	<i>Capsella bursa-pastoris (L.) Medik.</i>	T	R	4
CHEAL	<i>Chenopodium album L.</i>	T	CR	7
CONAR	<i>Convolvulus arvensis L.</i>	G	CR	5
CYPES	<i>Cyperus esculentus L.</i>	G	C	5
DACGL	<i>Dactylis glomerata L.</i>	H	C	6
DAUCA	<i>Daucus carota L.</i>	H	CR	4
DICXX	Dicot unknown	NA	NA	NA
EQUAR	<i>Equisetum arvense L.</i>	G	CR	3
HELTU	<i>Helianthus tuberosus L.</i>	G	C	6
KICSP	<i>Kickxia spuria (L.) Dumort.</i>	T	SR	4
LOLMU	<i>Lolium multiflorum Lam.</i>	T	C	6
MATMA	<i>Anthemis arvensis L.</i>	T	R	5
MEDSA	<i>Medicago sativa L.</i>	H	C	3
MONXX	Unknown monocot	NA	NA	NA
PHACA	<i>Phalaris canariensis L.</i>	NA	NA	NA
PICEC	<i>Picris echioides L.</i>	T	CSR	2
PLALA	<i>Plantago lanceolata L.</i>	H	SR	NA
POAAN	<i>Poa annua L.</i>	T	R	8
POLAV	<i>Polygonum aviculare L.</i>	T	R	1
RUMCR	<i>Rumex crispus L.</i>	H	C	5
SCONX	Unknown dicot two	NA	NA	NA
SETVI	<i>Setaria viridis (L.) Beauv.</i>	T	CR	7
SOLNI	<i>Solanum nigrum L.</i>	T	CR	7
SONAR	<i>Sonchus arvensis L.</i>	H	CR	NA
STEME	<i>Stellaria media (L.) Vill.</i>	T	CR	8
TRFAR	<i>Trifolium arvense L.</i>	T	SR	1
VICXX	<i>Vicia villosa Roth</i>	T	CR	NA
XANST	<i>Xanthium strumarium L.</i>	T	CR	6

Annex 4: Species Bayer code used in NMDS analysis, latin name and characteristic considered in species composition analysis. ¹ Raunkiaer life form for herbaceous species (Raunkiaer, 1934). G, Geophytes; T, Terophytes; H, Hemiciptophytes. ² Grime (Grime, 1979) plant strategy. C, competitive; S, stress tolerant; R, Ruderal. ³ Ellenberger indicator values (Pignatti et al. 2005) for soil fertility (N).

(a)

(b)

Annex1: Precipitation and temperature (max e min) reported each ten days, during the maize growing period in 2011(a) and 2012(b), measured in San Piero a Grado (PI).

Annex 2: Linear correlation between: number of leaf (n.leaf), Leaf area index (LAI), length of leaf (Length), average leaf width (Av.Width). Data reported where collected in 2011 (58 days after sowing) and in 2012 (34 and 45 days after sowing)). In the upper plot is reported the R^2 , in the lower part the scatter plot. For R^2 higher than 0.372 $P < 0.01$.

Annex 3: GM effects on time (day after sowing), collected in 2012, clock wise starting from upper left maize height (cm), phenological phase according to the BBCH scale, SPAD values on the 5th leaf, SPAD values on the 4th leaf. The values for GM+ are represented in blue green, GM- in red.

EPPO Code	Latin Name	Raunkiaer ¹	Grime strategy ²	Ellemberg N values ³
AGRRE	<i>Agropyron repens (L.) Beauv.</i>	G	CS	8
AMARE	<i>Amaranthus retroflexus L.</i>	T	CR	9
AMIMA	<i>Ammi majus L.</i>	T	CR	2
ANAGAR	<i>Anagallis arvensis L.</i>	T	R	6
BEAVU	<i>Beta vulgaris L.</i>	H	CS	5
CAGSE	<i>Calystegia sepium (L.) R. Br.</i>	H	CR	9
CAPBP	<i>Capsella bursa-pastoris (L.) Medik.</i>	T	R	4
CHEAL	<i>Chenopodium album L.</i>	T	CR	7
CONAR	<i>Convolvulus arvensis L.</i>	G	CR	5
CYPES	<i>Cyperus esculentus L.</i>	G	C	5
DACGL	<i>Dactylis glomerata L.</i>	H	C	6
DAUCA	<i>Daucus carota L.</i>	H	CR	4
DICXX	Dicot unknown	NA	NA	NA
EQUAR	<i>Equisetum arvense L.</i>	G	CR	3
HELTU	<i>Helianthus tuberosus L.</i>	G	C	6
KICSP	<i>Kickxia spuria (L.) Dumort.</i>	T	SR	4
LOLMU	<i>Lolium multiflorum Lam.</i>	T	C	6
MATMA	<i>Anthemis arvensis L.</i>	T	R	5
MEDSA	<i>Medicago sativa L.</i>	H	C	3
MONXX	Unknown monocot	NA	NA	NA
PHACA	<i>Phalaris canariensis L.</i>	NA	NA	NA
PICEC	<i>Picris echioides L.</i>	T	CSR	2
PLALA	<i>Plantago lanceolata L.</i>	H	SR	NA
POAAN	<i>Poa annua L.</i>	T	R	8
POLAV	<i>Polygonum aviculare L.</i>	T	R	1
RUMCR	<i>Rumex crispus L.</i>	H	C	5
SCONX	Unknown dicot two	NA	NA	NA
SETVI	<i>Setaria viridis (L.) Beauv.</i>	T	CR	7
SOLNI	<i>Solanum nigrum L.</i>	T	CR	7
SONAR	<i>Sonchus arvensis L.</i>	H	CR	NA
STEME	<i>Stellaria media (L.) Vill.</i>	T	CR	8
TRFAR	<i>Trifolium arvense L.</i>	T	SR	1
VICXX	<i>Vicia villosa Roth</i>	T	CR	NA
XANST	<i>Xanthium strumarium L.</i>	T	CR	6

Annex 4: Species Bayer code used in NMDS analysis, latin name and characteristic considered in species composition analysis. ¹ Raunkiaer life form for herbaceous species (Raunkiaer, 1934). G, Geophytes; T, Terophytes; H, Hemicriptophytes. ² Grime (Grime, 1979) plant strategy. C, competitive; S, stress tolerant; R, Ruderal. ³ Ellenberger indicator values (Pignatti et al. 2005) for soil fertility (N).