

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

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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

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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:

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

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

13

Results and discussion

Maize potential competitive ability

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

According to the Friedman rank sum test, the phenological phase of maize in 2011 was significantly ($P < 0.05$, Friedman's chi-squared statistic = 7.69) more advanced in plots where green manure had been applied (median BBCH = 35) compared to plots where GM was not applied (median BBCH = 33), while in 2012 no significant effect was detected (see Annex 3). With respect to P application, no significant effects on crop 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).

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 that P application, in the form of P-enriched compost, does not affect the magnitude of weed emergence, unlike Blackshaw and Molnar (2009) who found a significant effect of P application. However, these authors did not use 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 particularly important in case of high presence of weed species emerging at the same time as maize, thus potentially more competitive (e.g. *Xanthium strumarium* L., *Datura stramonium* L., *Echinocloa crus-galli* L., *A. retroflexus*, *Sorghum halepense* L., *Chenopodium album* L.). This could lead to a potentially high yield reduction (Gołębiowska and Kieloch, 2016; Yousefi *et al.*, 2015).

17

18 **Maize/weed competition**

When considering the effect of green manure on weed and crop competition, neither the RRI or RCI indices showed any significant interaction between green manure and P fertilization (Chi-square:1.446 and 2.515 for RRI_c in 2011 and 2012; 1,102 for RRI_w in 2012, and 1.562 for RCI in 2012). In both years, the results of the Z-test on RRI clearly indicated a competitive advantage for maize when grown after green manure (RRI_c < 0), 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

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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.