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

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Abstract

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 an appropriate level of satisfactory crop yields. We studied how green manuring with hairy vetch (*Vicia villosa* Roth.) and the application of different types of phosphorous-enriched compost affect weed/maize (*Zea mays* 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 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, favoring maize, but also creating favorable conditions for unwanted weed species such as competitive-ruderals. Increasing nitrogen availability in the early growth stages of maize through green manuring can increase crop competitive ability. However, 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.

Introduction

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

In organic farming, weed control in maize can be extremely challenging and relies on an Integrated Weed Management (IWM) strategy through the application of both direct (e.g., mechanical tools or flaming) and indirect methods (Bàrberi, 2002). Indirect methods include all crop practices that prevent high weed occurrence while simultaneously stimulating crop growth and competitive ability. In this context, nitrogen (N) and phosphorus (P) fertilization may play a crucial role (Grant *et al.*, 2001; Rajcan and Swanton, 2001). In fact, crop management recommendations rarely consider that added nutrients might enhance weed growth as well as crop growth, resulting in crop/weed competitive interactions which are potentially detrimental for the crop yield (Blackshaw *et al.*, 2004; Blackshaw and Brandt, 2008). Information on how each nutrient type affects crop/weed competition might help to improve IWM systems. N and P availability may affect the maize capacity to compete with weeds, by favoring the expression of plant traits that are more related to a higher competitive ability, e.g., leaf area and plant height. Mohammadi (2007) showed that leaf area is one of the most reliable parameters to estimate maize competitive ability against weeds, while Zystro *et al.* (2012) found that plant height was the best predictor for estimating the weed suppressive ability of sweet maize varieties.

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

Weed suppression and nutrient (especially N) release are two of the many benefits potentially provided by green manures. Legume green manures, such as hairy vetch (*Vicia villosa* Roth.), supply N to the soil through symbiotic N_2-fixation and increase soil P availability by releasing acid root exudates (Karasawa and Takahashi, 2015) and/or hosting symbioses with arbuscular mycorrhizal fungi (AMF) (Njeru *et al.*, 2014). Hairy vetch is one of the most efficient N-fixing crops (e.g., Hartwig and Ammon, 2002), which accumulates a large amount of N during its growing cycle (Anugroho *et al.*, 2009). About 50% of this N is readily available for the subsequent cash crop after soil incorporation (green manuring) (Brandsæter *et al.*, 2008), but also for the field weed community (Bittencourt *et al.*, 2013).

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

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

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

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

Materials and methods

To test our three hypotheses, a field trial was established at the Centre for Agri-Environmental Research 'Enrico Avanzi' (CiRAA) of the University of Pisa (Italy) in 2010–2011 and 2011–2012. The area has a coastal Mediterranean climate with a mean annual precipitation of 844 mm and a mean annual temperature of 15°C. Precipitation is mainly in autumn and early spring, whilst summers are usually dry. In the two experimental years precipitation was exceptionally low (Annex 1), especially in 2011, with negative effects on biomass production of cash crops and weeds.

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

The experimental layout was a split-plot design with three replications, with a sub-plot size of 8×8 m^2. The main plots were characterized by either the presence (GM+) or absence (GM−) of a winter green manure.

Hairy vetch *cv.* Latigo was broadcast seeded at a rate of 100 kg ha^{-1} on 15 September 2010 and 31 October 2011, in the first and second year, respectively. The relatively high seeding rate (100 kg ha^{-1}) was chosen to promote an adequate above ground yield biomass. No fertilization, crop protection or direct weeding measures were applied on hairy vetch until termination, which was done by disc harrowing at the flowering stage (8 April 2011 and 24 April 2012). Averaged across all plots, the biomass produced by the vetch accounted for 1.2 (S.D. = 0.21) t ha^{-1} of dry matter in 2011 and 5.3 (S.D. = 0.99) t ha^{-1} in 2012, corresponding to a N supply of 27.4 and 194 kg ha^{-1}, respectively. The sub-plots included six different P fertilization (PF) treatments: an unfertilized control (C^{-}); rock phosphate (C^{+}a); a green compost amended with rock phosphate powder at the beginning of compost production, at a rate of 100 kg t^{-1} fresh matter (EP); a compost obtained from the same raw material as EP but not enriched in P (NEP); and rock phosphate and a non P-enriched compost (C^{+}b) before maize sowing. All the composts were produced by the International Center for High Mediterranean Agronomic Studies (CIHEAM—IAM, Bari, Italy) using the same raw material (clippings from lawns, ornamental palms and olives) in EP, NEP and C^{+}b in both years (Bustamante *et al.*, 2016; Mihreteab *et al.*, 2016; Ciaccia *et al.*, 2017). Except the control (C^{-}), all treatments included an amount of compost or rock phosphate powder calculated to replenish the P deficit of the 5-year crop rotation, estimated as 24 kg ha^{-1} (unpubl. obs.). Compost treatment and rate of application are reported in Table 1.

Table 1. P fertilization treatment application rates expressed as total materials and total P_2O_5

Treatment	Compost factor	Compost (kg ha^{-1})	P-enriched compost (kg ha^{-1})	Rock phosphate (kg ha^{-1})	Total P_2O_5 (kg ha^{-1})
C^-	Untreated (No compost, no rock phosphate)	0	0	0	0
C^+a	Rock phosphate	0	0	204	24
C^+b	Compost + rock phosphate	3619	0	158	24
EP	P-enriched compost	0	3478	0	24
NEP	Not P-enriched compost	16,250	0	0	24

Maize (cultivar PR36Y03, Pioneer FAO class 300) was sown on 18 April 2011 and 15 May 2012, respectively, in the first and second years, at a rate of 80,000 seeds ha^{-1}. Maize phenology was assessed from the early stages to flowering using the BBCH scale (Lancashire *et al*., 1991). Most measurements were taken during the critical periods for maize/weed competition, i.e., from 3- to 14-leaf stage according to Hall *et a*l. (1992). Maize plant height, phenological stage, number of leaves, mean leaf width, length and area were measured on three plants plot^{-1} 58 days after sowing (DAS) (BBCH scale from 31 to 37, mean = 34). In 2011 these data were used for a preliminary assessment of the potential competitive ability of maize. In 2012, a higher number of measurements were taken to obtain better estimates: plant height and phenological phase were assessed 24, 28, 32, 43, 50, 58, 64, 71 and 90 days after maize sowing, corresponding to mean BBCH values of 15, 16, 18, 31, 32, 35, 52, 60 and 74, respectively. A number of leaves per plant, mean leaf width, length and area, reported as Leaf Area Index (LAI), were measured 34 and 54 DAS. Crop N nutritional status was measured by a chlorophyll meter (SPAD-502, Konica Minolta Holding, Inc.) at 24, 34, 43 and 50 DAS on the fourth and fifth leaves. Total maize biomass was measured only at harvest in 2011, and at 28, 34, 43 DAS in 2012.

Weed density by species was measured before post-emergence inter-row cultivation (hoeing), which happened only once in the 2 years when maize had five unfolded leaves (BBCH 15). Weed density was assessed on three 50 × 50 cm^2 sampling areas plot^{-1} on 20 May 2011 and 8 June 2012, respectively. In 2011, weed biomass was very limited throughout the maize cropping cycle due to drought and thus was not sampled. In 2012, three weed biomass samplings were performed at 28, 34 and 43 DAS, respectively.

Statistical analysis

Maize potential competitive ability

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

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

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

Weed density and weed community diversity

Using weed density data, the following parameters were calculated: species richness, the Shannon diversity index (HS) (Magurran, 1988), the inverse Simpson index of diversity (invsimp) (Peet, 1974), and Pielou's evenness index (J) (Sheldon, 1969). Weed density was partitioned into functional groups upon the following response traits: (1) Raunkiær life form for herbaceous species (Raunkiaer, 1934), (2) Grime plant strategy (Grime, 1979), (3) two groups based on Ellenberg indicator values for soil fertility (N) (Pignatti *et al*., 2005): one group composed of species with higher value than the mean of all species present, and the other composed of species with lower values than the mean. To separate the effect of the tested factors on functional groups from their general effect on overall weed density, we calculated the relative density by functional group (sum of the densities of all species belonging to a functional group divided by total weed density, expressed as a percentage). Species richness, diversity indices and relative density of functional groups were considered as independent variables in a split-plot design ANOVA, in a Mixed Effect Model, with a Poisson distribution for weed density and species richness data, Gaussian distribution for HS, invsimps and J, and binomial distribution for relative density by functional group. Tukey's *post-hoc* test was applied where needed.

Weed community composition

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

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

$$RCI = RRI_w - RRI_c$$

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 the 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.

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

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

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

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

Among all maize parameters considered, the green manure application showed a clear and consistent effect in enhancing plant height and LAI, which are two of the most 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 potential competitive ability of maize.

Weed density, biomass and diversity

Phosphorus fertilization ($P < 0.001$, $\chi^2 = 65.33$, Df = 4) and green manure × P fertilization interaction ($P < 0.01$, $\chi^2 = 33.36$, 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

Table 2. Maize leaf area, height and biomass in 2011 and 2012 in the different green manure and phosphorus fertilization (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 × 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

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

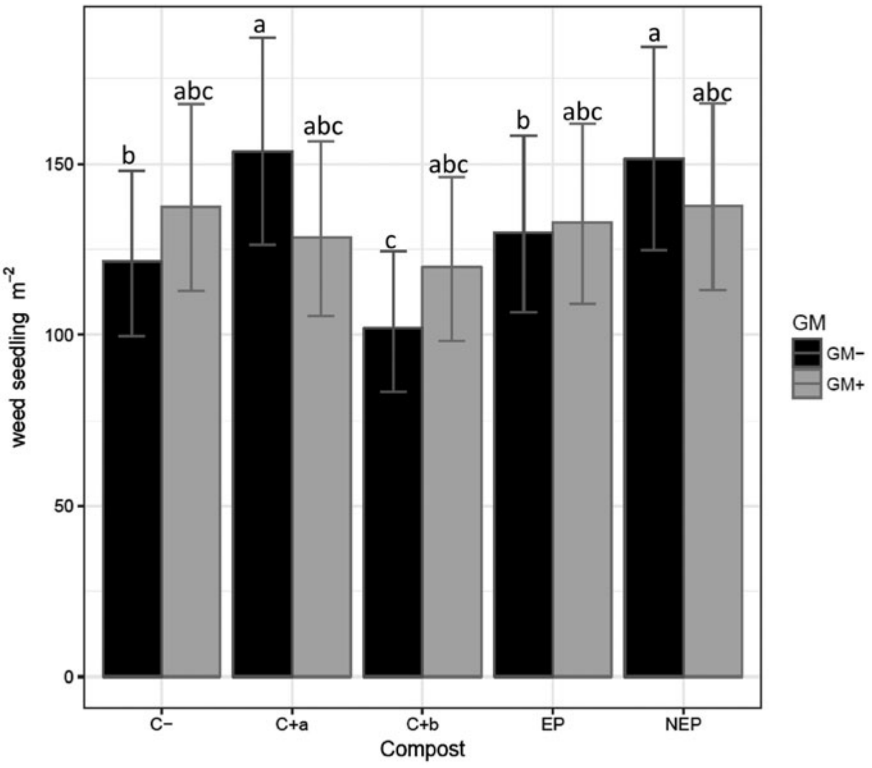

Fig. 1. Total weed density as compost × green manure (GM) interaction; error bars represent the standard error (s.e.). GM presence is reported in grey, GM absence in black. (C−, untreated −no compost, no rock phosphate-; C+a, rock phosphate only; C+b, Compost + rock phosphate; EP, P enriched compost; NEP, compost only; composition and rate of application are reported in Table 1).

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

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

	2011		2012	
Years	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 × PF	0.277	0.109	0.224	0.069
Total	2.524		2.727	

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

Weed community composition

According to the results of the permutational multivariate analysis of variance (Table 3), green manure was the only factor that significantly affected weed community composition at an early stage in both years ($P < 0.01$), the magnitude of the effect was 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 (Figs. 2a and 2b). Species affinity with green

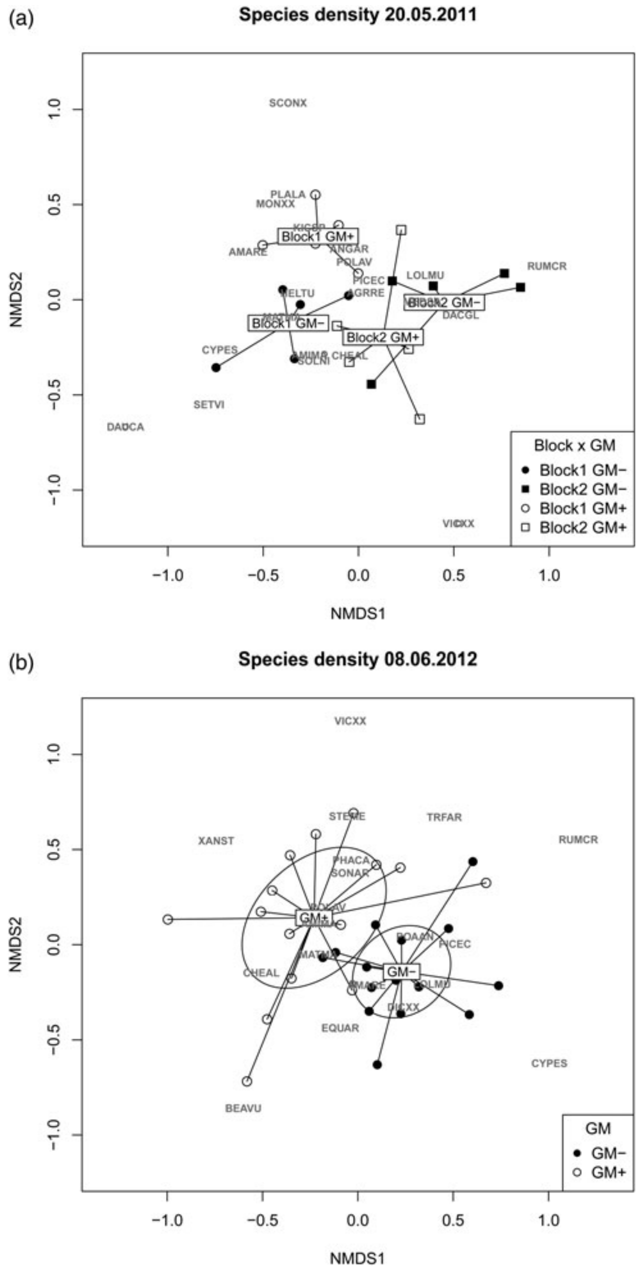

Fig. 2. (a) Site ordination (NMDS) based on floristic similarities of 20 plots ($k = 2$, non-metric fit: $R^2 = 0.953$, stress = 0.215). GM, Green manure. Species names are reported in Annex 4. (b) Site ordination (NMDS) based on floristic similarities of 30 plots ($k = 2$, non-metric fit: $R^2 = 0.939$, Stress: 0.246).GM, Green manure. Species names are reported in Annex 4.

manure application was consistent between years (compare Figs. 2a and 2b). In particular, green manure selected for annual nitrophilous dicotyledonous species (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 annual grasses such as *Lolium multiflorum* Lam. and perennial species such as *Cyperus esculentus* L. 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.

Table 4. Effect of green manure, phosphorus fertilization (PF) and green manure × PF interaction on the relative density of Ruderals and Competitive-Ruderals weeds

	Ruderals (%)		Competitive-Ruderals (%)	
	2011	2012	2011	2012
Green manure	**	*	NS	*
χ^2	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
χ^2	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 × PF	NS	NS	NS	NS
χ^2	2.697	1.476	1.594	2.075

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

Table 5. Effect of green manure on Relative Response Index of crop (RRIc) and weeds (RRIw) and Response Comparison Index (RCI) in 2011 and 2012

	RRIc		RRIw[a]	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

[a] In 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.

Weed community functional analysis

In accordance with the Raunkiær 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 Raunkiær life forms was never significantly affected by the experimental factors. In 2012, due to a very high incidence of therophytes (in 75.6% of the samples, the only species present were therophytes), no such analysis was performed. Regarding Grime's plant strategy groups, the

Table 6. Effect of Phosphorus fertilization on Relative Response Index of crop (RRIc) and weeds (RRIw) and Response Comparison Index (RCI) in 2011 and 2012

	RRIc		RRIw (2012)#			RCI (2012)#		
PF effect[a]	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**
Green manure × PF interaction[b]								
Significance	NS	NS	NS	*	NS	NS	*	(*)
χ^2	2.139	1.086	1814	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

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.

PF, phosphorus fertilization (mean of all treatments but the control); GM+, application of green manure; GM−, plots without green manure.

(*),*Significant at $P < 0.10$ and 0.05, respectively.

[a] Error DF = 3.

[2] Error DF = 1.

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 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).

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 (χ^2:1.446 and 2.515 for RRI_C in 2011 and 2012; 1102 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 significantly higher or lower than 0), as reported in Table 5. As a consequence, the RCI for the green manure factor showed that crop/weed competitive relationships favored the crop to the detriment of weeds when green manure was applied.

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

The RRI and RCI indices that were calculated to study the effect of the P fertilization factor on crop/weed competition (Table 6) also indicated a competitive advantage for the cash crop (negative RRI_c and positive RRI_w) when P was applied. However, the way P was applied did not significantly affect the results (χ^2: 0.655 and 3.876 for RRI_c in 2011 and 2012; 7.211, 8.039 and 1.442 for RRI_w at 28, 34 and 43 DAS, respectively, in 2012; 2.535, 9.414 and 0.447 for RCI at 28, 34 and 43 DAS, respectively, in 2012). In contrast, RRI_w and RCI calculated at 34 DAS in 2012 revealed that weeds were more competitive when P was applied together with green manure, and that maize was more competitive when P was applied alone (Table 6). Although this effect was found just in one of the three sampling dates, when N and P are more available for weeds they may stimulate weed growth even in a quite N-demanding crop like maize (in accordance with Di Tomaso, 1995 and Davis and Liebman, 2001). However, in our study the overall effect of green manure and P fertilization was to increase the competitive ability of maize against weeds.

Conclusions and recommendations

The data presented in this paper confirm that the complexity of interactions between soil fertility, crop and weeds can be steered towards a crop benefit by implementing agronomic techniques that increase soil fertility whilst keeping weeds under control.

Combining high N_2-fixing legume green manures with P-enriched amendments can promote nutrient cycling in organic stockless cropping systems, where the absence of farmyard manure may lead to a shortage of soil N and P in the long term. Nevertheless, in our conditions there was no immediate effect of P-enriched compost on early maize growth. Likewise, the application of green manure and/or P enriched compost did not increase early stage weed infestation in maize. We argue that the dry conditions might have indeed slowed down the mineralization rate of the green manure and of the composts, leading to no immediate availability of N, and especially P, for either maize or weed plants. Further research under more humid spring conditions is thus required to unravel the potential of these two nutrient sources of suppressing weeds in maize. Soil incorporation of a hairy vetch green manure clearly enhanced the expression of competitive traits of maize (i.e., plant height and LAI) and, overall, the competitive ability of maize against weeds. On the other hand, green manure shifted weed community composition towards a higher relative abundance of ruderal and competitive-ruderal species. These dynamics should be monitored if these species become dominant, in which case dedicated management tactics would need to be implemented. We found that hairy vetch green manure reduced the development of weed species groups such as creepers and perennials (e.g., *C. esculentum*), which are often detrimental to maize production. Overall, the weed community shifts induced by green manure seem to lead to a less aggressive weed community for subsequent spring-summer crops. However this needs to be confirmed in a longer-term perspective, in particular considering the very dry conditions of the 2 years studied during our tests.

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