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# First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth.

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# First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth

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## Abstract

Arbuscular mycorrhizal fungi are increasingly used in organic cropping systems to increase yields. Although cover crops are largely used in organic farming, there is little knowledge on the impact of cover crops on native mycorrhizal fungi. Here we studied the effect of cover crop diversity on mycorrhizal colonization in subsequent organic maize cultivars differing in the level of genetic diversity. Experiments were conducted from 2010 to 2012 in a Mediterranean environment. First Indian mustard (*Brassica juncea* L. Czern.), hairy vetch (*Vicia villosa* Roth), a mix of seven cover crop species (Mix) and natural vegetation (Control) were cultivated as winter cover crops. Then an organically and a conventionally bred maize hybrid, and three organically bred composite cross populations were cultivated. Mycorrhizal propagule dynamics were measured. Results at juvenile stage show a higher mycorrhizal colonization in maize plants grown after hairy vetch, of 35.0%, and Mix cover crops, of 29.4%, compared to Indian mustard, of 20.9%, and Control, of 21.3%. The potential of soil mycorrhization decreased of 56.5% following Indian mustard, higher than that of other cover crops, of 34.1-47.3%. This finding could be explained by the release of isothiocyanates in soils. Moreover, maize shoot biomass, nitrogen and phosphorus content across all maize genotypes at juvenile stage increased with mycorrhizal colonization. These findings provide the first evidence of the greater role played by cover crop identity in the enhancement of early mycorrhizal colonization of the subsequent crop and of soil mycorrhizal activity.

**Keywords** Cover crops – Organic agriculture – Arbuscular mycorrhizal fungi – Maize genotypes – Crop diversity – Mycorrhizal inoculum potential

## 1 Introduction

Beneficial soil biota provide essential ecological services and represent key elements of soil fertility and productivity in organic farming systems (Pimentel et al. 1997). Arbuscular mycorrhizal fungi (AMF) belong to one of the most important groups of beneficial soil biota, establishing mutualistic symbioses with the roots of most land plants, including the large majority of agricultural crops (Smith and Read 2008). AMF deliver many essential agroecosystem services, such as nutrient uptake, soil aggregation and carbon sequestration (Gianinazzi et al. 2010), by means of an extensive extraradical hyphal network spreading from colonized roots into the soil (Avio et al. 2006; Fortuna et al. 2012) and have been regarded as 'agroecosystem engineers' (Rinaudo et al. 2010). In addition, AMF increase plant resistance to biotic and abiotic stresses (Smith and Read 2008) and affect the synthesis of beneficial plant secondary metabolites, contributing to the production of safe and high quality food (Giovannetti et al. 2012).

AMF exploitation as biofertilisers has been implemented by the deliberate release of exotic strains into agroecosystems (Gianinazzi et al. 2010). Less attention has been focused on the possibility of raising inoculum potential of AMF indigenous strains by appropriate agricultural management practices. Such a strategy would be fundamental in low-input and organic farming, which rely more on agroecological approaches than on the use of external inputs. Enhancement of indigenous strains would promote early colonization of field crops, increasing the expression of agroecosystem services (Bittman et al. 2006).

Cover crops are widely recognized as an important management practice for sustainable agriculture because of their contributions to soil conservation and quality, and to crop performance (Kabir and Koide 2002; Weil and Kremen 2007). They have been reported to help maintain or increase mycorrhizal potential of soils, e. g. providing nourishment during winter periods to AMF, which are obligate mutualists (Kabir and Koide 2002). When the agricultural fields lie fallow through the winter season, AMF populations are deprived of carbohydrates, and consequently are considerably reduced by the start of the next cropping season. Thus, mycotrophic cover crops may be fundamental in maintaining a high inoculum potential in the absence of the cash crop during seasonal fallow periods.

Nonetheless, some cover crops - mainly members of the Brassicaceae family - are not mycorrhizal, and may reduce AMF colonization in the subsequent crop. Some studies have indicated reduced mycorrhizal colonization of the subsequent crop after the growth of a *Brassica* species (Gavito and Miller 1998; Koide and Peoples 2012) while others did not report any change (Pellerin et al. 2007; White and Weil 2010). Thus, to delineate how cover crops influence field AMF populations it would be necessary to have comparative field experiments that encompass both AMF host and non host cover crops.

In short season crops, such as maize, AMF benefit may depend on early and large root colonization, which in turn is strictly correlated with soil inoculum potential (Bittman et al. 2006). Mycorrhizal dependency and responsiveness also depend on plant genotypes, which vary among different

crops (Tawaraya 2003; An et al. 2010). Plant breeding to create novel genotypes more efficient in nutrient and water resource use represents a key target for sustainable agriculture. Crop breeding is generally carried out in research stations where nutrients are not a limiting factor, possibly leading to the production of hybrids less responsive to AMF. By contrast, breeding programs in organic agriculture should focus on crop genotypes that make sustainable use of the available soil bioresources (Wolfe et al. 2008). Thus, a profitable use of AMF in an organic and low-input farming context, will require the selection of a suitable combination of plant host, fungal partner and agricultural management practices (Sawers et al. 2008).

Here, we tested the hypothesis that increasing the genetic (breeding) and species (cover crop) diversity will provide a more favorable environment for AMF activity in an organic system (Fig. 1). The specific aims of this study were: i) to assess the effects of three winter cover crop treatments, differing in species diversity, and fallow on AMF colonization of five subsequent maize crop genotypes at the juvenile stage and at harvest; ii) to monitor the effects of three winter cover crop treatments and fallow on soil mycorrhizal potential; iii) to examine the growth responses of maize plants at juvenile stage and their relationship with early mycorrhizal colonization; iv) to assess AMF susceptibility of two maize hybrids (organically and conventionally bred) compared with three composite cross populations (organically bred) of higher genetic diversity, at the juvenile stage and at harvest.

## 2 Materials and methods

### 2.1 Experimental site

The experimental fields were located at the Interdepartmental Centre for Agri-environmental Research "Enrico Avanzi" (CIRAA) of the University of Pisa, located at S. Piero a Grado, Pisa (latitude 43°40' N, longitude 10°18' E) in Italy. The fields are part of a long-term experimental system, MASCOT (Mediterranean Arable Systems Comparison Trial) established in autumn 2001, comparing organic and conventional management systems for a 5-year stockless arable crop rotation (Mazzoncini et al. 2010). Physical and chemical characteristics of soil are: clay, 19.4%; silt, 29.2%; sand, 51.4%; pH (water) 8.3, total organic carbon, 9.3 g kg $^{-1}$, total N, 1.1 g kg $^{-1}$, and available P (Olsen analysis), 6.7 g kg $^{-1}$. The crop rotation includes maize (*Zea mays* L.), common wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), pigeon bean (*Vicia faba* L. var. *minor*) and durum wheat (*Triticum durum* Desf.). The experiment embeds additional organically-managed fields ('organic playgrounds') where specific plot experiments are allocated (Bàrberi and Mazzoncini 2006).

### 2.2 Experimental design

The experiment was laid out in one organic playground as a split plot design with three blocks, and in each year it was performed in a different field. Main plots included four soil cover treatments, namely *Brassica*

*juncea* (L.) Czern. cv. ISCI 20 (Indian mustard), *Vicia villosa* Roth cv. Latigo (hairy vetch), a mix of seven species (hereafter called 'Mix') and a no-till fallow with natural vegetation (hereafter called 'Control'). The Mix treatment, supplied as a commercial mixture by Arcoiris s.r.l. (Modena, Italy), included: *Fagopyrum esculentum* Moench (buckwheat), *Lupinus albus* L. (white lupin), *Phacelia tanacetifolia* Benth. (lacy phacelia), *Pisum sativum* L. (common pea), *Trifolium alexandrinum* L. (berseem clover), *Trifolium incarnatum* L. (crimson clover) and *V. villosa*. Subplots included five maize genotypes, two hybrids (Pioneer® PR64Y03 and MvTC TO341, developed under conventional and organic management respectively) and three composite cross populations, namely Complete Composite, Composite 1 Gyula and PC Composite. Composite cross populations are populations of segregating individuals formed by inter-crossing seed stocks with divergent evolutionary origins, followed by bulking and propagation of the F1 progenies in successive cropping seasons (Phillips and Wolfe 2005). Compared to hybrids, they are thus characterised by higher genetic diversity. Composite cross populations and the organic hybrid seeds were provided by the Centre for Agricultural Research, Agricultural Institute, Hungarian Academy of Sciences, Martonvásár. The whole trial was then composed of 60 subplots each measuring 3 × 10 m.

2.3 Cover crop management

Cover crops were sown on 18 October 2010 at a seeding rate of 9 kg $ha^{-1}$ (*B. juncea)*, 100 kg $ha^{-1}$ (*V. villosa*) and 50 kg $ha^{-1}$ (Mix). In 2011, cover crops were sown on 19 October at higher rates, since cover crop biomass in the previous year was lower than expected and to ensure adequate plant stand: 12 kg $ha^{-1}$ (*B. juncea*), 120 kg $ha^{-1}$ (*V. villosa*), and 65 kg $ha^{-1}$ (Mix). Weeds were not controlled in any of the treatments. Cover crops and weeds were sampled on 21 April 2011 and 23 April 2012 from four randomly selected 0.25 $m^2$ quadrates $plot^{-1}$. Cover crop and weeds were separated and oven dried at 80°C until constant weight. Total shoot dry biomass (cover crop and weeds) ranged from 165 g $m^{-2}$ in Control to 200 in *B. juncea*, 400 in *V. villosa* and 440 in Mix in 2011, and from 750 g $m^{-2}$ in *B. juncea* to 800 in *V. villosa*, 900 in Mix and 920 in Control in 2012, weeds representing about 20-60% and 40-70% of the total biomass in 2011 and 2012, respectively. In particular, in *B. juncea* weeds represented 64% and 47% of the total biomass. The dominant weeds were represented by the AMF hosts *Lolium* spp., *Cynodon dactylon* (L.) Pers. and *Avena* spp., which occurred as natural vegetation in Control treatment. No differences in weeds distribution were observed among treatments. Each year, cover crops were mown at the end of April and immediately incorporated into the soil by disc harrowing at a depth of 15 cm.

2.4 Maize sowing and management

Maize genotypes were sown on 26 April 2011 and 5 June 2012 at a spacing of 50 × 28 cm. Delayed sowing in 2012 was due to prolonged heavy rain and cold. Nutex Letame (Sipcam Italia S.p.A., Pero, Italy), a

pelleted mixture of selected manures (NPK=3:3:3), was applied only in 2011 at 1000 kg $ha^{-1}$ rate as a starter fertiliser. Maize was grown as a rainfed crop, but in 2012 overhead irrigation was applied since an extremely dry and hot period occurred after the juvenile stage.

2.5 Plant sampling

Maize plants were sampled for AMF root colonization at the $4^{th}$ leaf (juvenile) phenological stage, and at final harvest stage. At juvenile stage (16 May 2011 and 2 July 2012) the sampling was done by uprooting 4 plants from each subplot, to recover the whole root system. The plants were placed in polythene bags and transported to the laboratory for analyses. Roots were processed for AMF assessment and shoots were oven dried at 60°C for 5 days, then weighed and preserved in sealed bags for N and P analyses. At harvest stage, 4 soil cores measuring about 8 cm in diameter and 15 cm in depth were obtained from the base of the sampled maize plants. The soil was washed through a 500 µm sieve to recover the roots.

2.6 Mycorrhizal root colonization of maize

At juvenile stage, maize roots were cleaned with tap water, cleared with 10% KOH in water bath at 80°C for 15 min, neutralized in 2% aqueous HCl and stained with 0.05% trypan blue in lactic acid. Root colonization was assessed under a dissecting microscope (Wild, Leica, Milano, Italy) at 25× or 40× magnification by the gridline intersect method (Giovannetti and Mosse 1980).

2.7 Mycorrhizal inoculum potential of the experimental field soil

Mycorrhizal inoculum potential (MIP) bioassay before sowing was carried out to verify the homogeneity of AMF propagules' distribution in the field soil. As *B. juncea* treatment reduced early AMF colonization in the subsequent maize crop, in the second year we decided to assess MIP on soil samples at different times, in order to investigate field AMF propagule density dynamics. Samples were taken: before cover crop sowing; at the end of cover crop cycle, a few days before soil incorporation; after soil incorporation of cover crops and tillage; at maize harvest. Soil samples (3 soil cores per subplot, taken 2.5 m apart at a depth of 5 to 15 cm) were dried, sieved using a 4 mm sieve and put in 50 ml tubes. Three replicated tubes were prepared for each MIP determination, for a total of 180 tubes. *Cichorium intybus* L. cv. Zuccherina di Trieste was sown in tubes put in transparent sun bags and maintained in a growth chamber at 27 °C and 16/8 h light/dark daily cycle until harvest. One week after germination plants were thinned to four per tube. Each tube was watered as needed. Plants were harvested 30 days after sowing and shoots excised and discarded. After removing the soil from tubes, roots were separated and cleaned with tap water. Roots were then cleared, stained and examined for AMF colonization assessment as described above.

171 2.8 Plant P and N uptake

172 P concentrations were measured after sulphuric/perchloric acid digestion using the photometric method,
173 whilst N concentrations were assessed using the Kjeldahl method. The total P and N contents were
174 calculated by multiplying P and N concentration values by dry weights.

175

176 2.9 Data analyses

177 Analyses of maize shoot dry matter, N and P content at juvenile stage were performed separately for each
178 year using a split-plot experimental design, since there was a significant interaction between genotype and
179 year. A mixed model with year as a random factor, cover crop and maize genotype as fixed factors was
180 adopted for soil MIP at the start of the experiment, maize AMF colonization at juvenile stage and harvest.
181 Pearson correlation coefficient was determined for maize shoot dry matter at juvenile stage *vs* AMF
182 colonization. The results of MIP bioassays for the second year were analysed by two way ANOVA, using
183 cover crop and time as factors, separately for each subsequent pair of sampling points. Percentage data
184 were arcsine transformed to fulfil the assumptions of ANOVA. Data reported in tables and figures were
185 then back transformed. Wherever feasible, a post hoc test was performed using Tukey's HSD test, while
186 orthogonal contrasts were used to test differences within hybrids and between hybrids and composite crop
187 population. All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

188

189 **3 Results and Discussion**

190 3.1 Maize mycorrhizal colonization at juvenile stage

191 MIP bioassay data showed no significant differences in AMF soil propagule density of the relevant
192 subplots at the start of the experiment (32.5-37.3% in the first year and 38.1-43.4% in the second year),
193 allowing us to consider mycorrhizal colonization data as only dependent on cover crop treatments and not
194 biased by a possible heterogeneous distribution of AMF propagules in the field. Mycorrhizal colonization
195 of maize at juvenile stage was significantly affected by cover crop treatments ($F_{3,12} = 5.41$, $p = 0.014$),
196 while it was not affected by year and genotype ($F_{1,2} = 0.81$, $p = 0.462$, and $F_{4,62} = 1.04$, $p = 0.394$). Maize
197 plants grown after *V. villosa* had the highest percentage of AMF colonised root length (35.0%±2.03%),
198 while plants grown after *B. juncea* and Control treatments had the lowest colonization levels (Fig. 2),
199 suggesting that *V. villosa*, as an AMF host plant, was able to sustain AMF natural communities better than
200 the non-host species *B. juncea* and fallow. The increased level of species diversity in Mix cover crop
201 treatment decreased AMF root colonization of maize, compared with *V. villosa*, indicating that cover crop
202 species functional identity (Costanzo and Bàrberi 2013) may play a more influential role than diversity in
203 determining the mycorrhizal status of the subsequent crop. In this experiment, we found a reduced level of
204 maize AMF colonization after *B. juncea* cover crop, in agreement with observations on oilseed rape

(*Brassica napus* L.) preceding maize (Koide and Peoples 2012). Our findings could be ascribed to a reduction, during the winter period, of AMF propagules, which, as obligate symbionts, depend on carbon sources supplied by host plants for their survival and on the maintenance of an extensive extraradical hyphal network able to boost mycorrhizal colonization of nearby plants (Giovannetti et al. 2004). Alternatively, the disruption and soil incorporation, as green manure, of *B. juncea* tissues, which contain glucosinolates producing biotoxic compounds, e. g. isothiocyanates after hydrolysis by myrosinase enzyme, may have had inhibitory effects on field AMF populations (Pellerin et al. 2007). Though, mycorrhizal colonization of maize grown after *B. juncea* did not differ from that obtained after fallow, as previously reported by other authors (Pellerin et al. 2007; White and Weil 2010). In our experimental system, the occurrence of host plant species growing as dominant weeds (*Lolium* spp., *Cynodon dactylon* (L.) Pers. and *Avena* spp.) may have buffered the negative effects of the non-host cover crop, maintaining soil mycorrhizal potential at the same level of the fallow treatment.

Maize genotypes did not significantly influence AMF colonization at juvenile stage in both years: all maize genotypes (both hybrids and composite cross populations) had a similar percentage of colonised root length (25.1 to 28.8%), suggesting that at juvenile stage soil mycorrhizal potential may play a more important role than genotype. Our results refer to the colonization of roots growing in the top soil layer (0-15 cm), since root colonization and propagules numbers decrease with depth (>20 cm) (Oehl et al. 2005).

### 3.2 Dynamics of soil mycorrhizal inoculum potential

Monitoring of AMF propagules over the growing season of cover crops and maize, as assessed by MIP, showed an interesting dynamics, with large variations depending on cropping system stages and related agronomic disturbance. MIP values at the end of cover crop cycle, before soil incorporation, were significantly higher than MIP values at cover crop sowing ($F_{1,104} = 20.9$; $p < 0.001$) (Fig. 4) independently from the cover crop treatments ($F_{3,6} = 0.25$; $p = 0.856$ for cover crop treatment and $F_{3,104} = 0.76$; $p = 0.517$ for interaction time × cover crop). Our data are consistent with previous data on soil inoculum potential obtained with hairy vetch as a winter cover crop (Galvez et al. 1995). However, results obtained with *B. juncea* treatment suggested that it did not affect the activity of AMF populations, possibly supporting our hypothesis on the role of AMF host weeds in buffering possible negative effects of non-host species.

A strong decrease of MIP values was detected after incorporation of cover crops into the soil (Fig. 4). Indeed, statistical analyses showed an effect of time ($F_{1,104} = 239.9$, $p < 0.001$). The significant interaction between cover crops and time ($F_{3,104} = 3.1$, $p = 0.029$) showed that MIP values after cover crop soil incorporation decreased differently depending on the type of cover crop, as confirmed by the Tukey's post hoc analysis following one way ANOVA performed on MIP data at this sampling time, which separated *B. juncea* from *V. villosa* and Mix. Several studies have reported the detrimental effects of tillage

on field AMF populations (Kabir 2005), although this aspect has not been extensively studied in cropping systems incorporating cover crops to increase soil fertility. Interestingly, there was a greater negative effect on MIP values of *B. juncea* cover crop, supporting our previous remarks on possible negative effects of isothiocyanates released by *B. juncea* tissues after soil incorporation.

At maize harvest, MIP values were higher than values after cover crop soil incorporation ($F_{1,104}$ = 583.2; p <0.001), due to a generalized increase, which varied depending on the cover crop treatment ($F_{3,6}$ = 6.14; p= 0.03 for cover crop treatment; $F_{3,104}$ = 3.15; p= 0.028 for time × cover crop interaction) (Fig. 4). Such a finding could be ascribed either to the growth of the host crop maize or to the favorable growing season (spring-summer, compared with fall-winter) promoting soil microbial biomass, AMF spore germination and spread of mycorrhizal networks in the soil (Gavito et al. 2002; Giovannetti et al. 2004).

3.3 Maize growth, N and P uptake at juvenile stage

Maize shoot dry matter at juvenile stage was significantly influenced by preceding cover crop ($F_{3,6}$=20.21, p = 0.002) and maize genotype ($F_{4,32}$=5.30, p = 0.002) in the year 2011 (Table 1), whereas in 2012 it was only affected by genotype ($F_{4,30}$= 2.84, p = 0.041). In 2011 both shoot N and P contents were significantly affected by cover crop treatments (p = 0.001 and 0.005 respectively) and genotypes (p = 0.014 and 0.017 respectively), while the interaction between the two was not significant (Table 1). Although cover crop effect was only statistically significant in 2011, its effect on maize shoot biomass, N and P uptake followed the same pattern in 2012: *V. villosa* > Mix > Control = *B. juncea* (Table 1), suggesting that *V. villosa* is a good winter cover crop for the subsequent summer crop, when used as green manure, representing a source of easily mineralisable N (Campiglia et al. 2010). In addition, *V. villosa*, as a $N_2$-fixing legume, can accumulate a large amount of N during the growing period, and make it available to the subsequent crop. The Mix treatment, containing species other than legumes, represents a less effective source of N than *V. villosa*. Therefore a better AMF colonization may have contributed to the uptake of the additional N available in soil (Hodge and Fitter 2010).

For each experimental year, we found a linear correlation between AMF root colonization and maize shoot dry matter production at juvenile stage ($r^2$=0.47, P <0.001, and $r^2$=0.29, P <0.001, in 2011 and 2012, respectively) (Fig. 3). Maize, being a relatively short-season crop, is known to benefit from an early and extensive mycorrhizal colonization both for juvenile growth and for grain yield at harvest (Bittman et al. 2006), as confirmed in our experiment where grain yield was higher in those cover crop treatments (*V. villosa* and Mix) which provide a higher early colonization level (N. Nol, personal communication).

3.4 Maize mycorrhizal colonization at harvest

At maize harvest, no significant differences in AMF root colonization among cover crop treatments were detected, consistently with earlier studies reporting that the reduced AMF colonization of maize after oilseed rape at the juvenile stage disappeared at silking (Gavito and Miller 1998). By contrast, percentage of mycorrhizal colonization was significantly affected by genotypes ($F_{4,64}$=2.67, p = 0.040), while no effect of cover crop×genotype interaction was found. Both maize hybrids showed a significantly lower AMF colonization (29.2-30.0%), than composite cross populations (32.8-33.1%) in both years, as revealed by orthogonal contrasts (p=0.002). However, the levels of colonization were high in both genotypes, confirming that modern hybrids do not necessarily show low levels of colonization (An et al. 2010).

## 4 Conclusions

Our experimental findings show that cover crops management affects soil mycorrhizal potential and early mycorrhizal colonization and growth of the subsequent maize crop. They also point out that choice of the right (i.e. most AMF supportive or less detrimental for AMF) cover crop species is more important than cover crop diversity (i.e. species mixture) in organic systems. Level of maize genetic diversity did not seem to influence AMF symbiosis to a great extent. In addition, the monitoring of AMF propagule dynamics over time evidenced that soil mycorrhizal potential values were negatively affected by soil incorporation of cover crops. Further investigations will elucidate whether the strong negative impact of *B. juncea* cover crop on AMF, reduced here by higher weed abundance under organic management, may be additionally alleviated by avoiding tillage and soil incorporation of Indian mustard biomass which could reduce the possible negative effects of isothiocyanates.

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373

374 **FIGURE LEGENDS**

375

376 **Fig. 1**. Maps and pictures showing the location of the experimental field where a split plot experiment
377 was laid out using four different cover crops [*Vicia villosa*, *Brassica juncea*, a mix of seven species
378 (Mix) and a no-till fallow (Control)], cultivated before five different maize genotypes [two hybrids
379 (Pioneer® PR64Y03 and MvTC TO341 developed under conventional and organic managements
380 respectively) and three Composite Cross Populations (Complete Composite, Composite 1 Gyula and PC
381 Composite)]. Arbuscular mycorrhizal structures (arbuscules and vesicles) were detected in the roots of the
382 different maize genotypes and in the roots of *Cichorium intybus* L. plants, which were used for the
383 mycorrhizal inoculum potential bioassay.

384

385 **Fig. 2** Maize AMF root colonization at juvenile stage, as influenced by the cover crop treatments:
386 *Brassica juncea*, no-till fallow (Control), a mix of seven species (Mix), and *Vicia villosa* during two years
387 experimental years. Note the higher levels of mycorrhizal colonization after the host species *V. villosa*
388 and the Mix treatment, compared with the non-host species *B. juncea* and Control. The same lower case
389 letters indicate no significant differences at $p \leq 0.05$ (Tukey's HSD test).

390

391 **Fig. 3** Relationship between percentage of AMF root colonization of maize and shoot dry matter at
392 juvenile stage (mg plant$^{-1}$) in 2011 ($r^2$=0.47; y=5.2x+62.3) and 2012 ($r^2$=0.29; y=13.7x+75.5)), showing
393 the impact of early mycorrhizal establishment on maize growth. As a relatively short-season crop, maize
394 may greatly benefit from an early and extensive AMF colonization. Each point represents data from
395 individual subplots.

396

397 **Fig. 4** AMF propagule dynamics as affected by cropping system stages, assessed by mycorrhizal
398 inoculum potential bioassay of the field soil. Sampling time (in days) were: 0 days: before sowing of
399 cover crop, 190 days: at the end of cover crop cycle before soil incorporation, 230 days: after cover
400 biomass soil incorporation and 350 days: at maize harvest. Note the strong decrease in AMF propagule
401 density after cover crop incorporation, which is higher in the non-host species treatment (*B. juncea*).
402 Vertical bars represent ± SE. When occurring within sampling times, different letters represent
403 statistically significant differences at $p < 0.05$ (Tukey's HSD test).

404

**Table 1.** Shoot dry matter, N and P content (mg plant$^{-1}$) of maize plants at juvenile stage, as influenced by cover crop and maize genotype treatments in 2011 and 2012.

| | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|
| | Shoot DM | N content | P content | Shoot DM | N content | P content |
| Cover crop | | | | | | |
| *V. villosa* | 317.0 c | 13.1 c | 0.95 c | 546.8 a | 17.9 a | 1.81 a |
| Mix | 231.9 b | 8.1 b | 0.73 b | 401.2 a | 11.0 a | 1.86 a |
| Control | 142.9 a | 4.0 a | 0.47 a | 347.0 a | 9.6 a | 1.75 a |
| *B. juncea* | 163.6 a | 4.7 a | 0.51 a | 367.5 a | 10.3 a | 1.53 a |
| Maize genotype | | | | | | |
| PR64Y03 | 258.1 b | 9.0 b | 0.76 b | 415.0 ab | 12.1 a | 1.80 a |
| MvTC TO341 | 159.9 a | 5.8 a | 0.50 a | 507.7 b | 14.3 a | 1.94 a |
| Complete composite | 216.2 ab | 7.4 ab | 0.67 ab | 364.3 a | 10.7 a | 1.60 a |
| Composite 1 Gyula | 182.2 ab | 6.5 ab | 0.61 ab | 414.8 ab | 12.7 a | 1.75 a |
| PC Composite | 252.8 b | 8.6 ab | 0.77 b | 365.0 a | 10.8 a | 1.58 a |
| *P* values of main factors and interaction | | | | | | |
| Cover crop | 0.002 | 0.001 | 0.005 | 0.534 | 0.384 | 0.984 |
| Maize genotype | 0.002 | 0.014 | 0.017 | 0.041 | 0.189 | 0.614 |
| Cover x Genotype | 0.847 | 0.477 | 0.618 | 0.169 | 0.263 | 0.486 |
| *P* values of linear orthogonal contrasts for maize genotype factor | | | | | | |
| Hybrids vs CCP | 0.637 | 0.885 | 0.361 | 0.034 | 0.134 | 0.236 |
| PR64Y03 vs MvTC TO341 | 0.001 | 0.003 | 0.005 | 0.039 | 0.143 | 0.499 |

Values followed by the same letter in a column within each treatment are not significantly different at P<0.05 (Tukey's HSD test)

2

Figure 3
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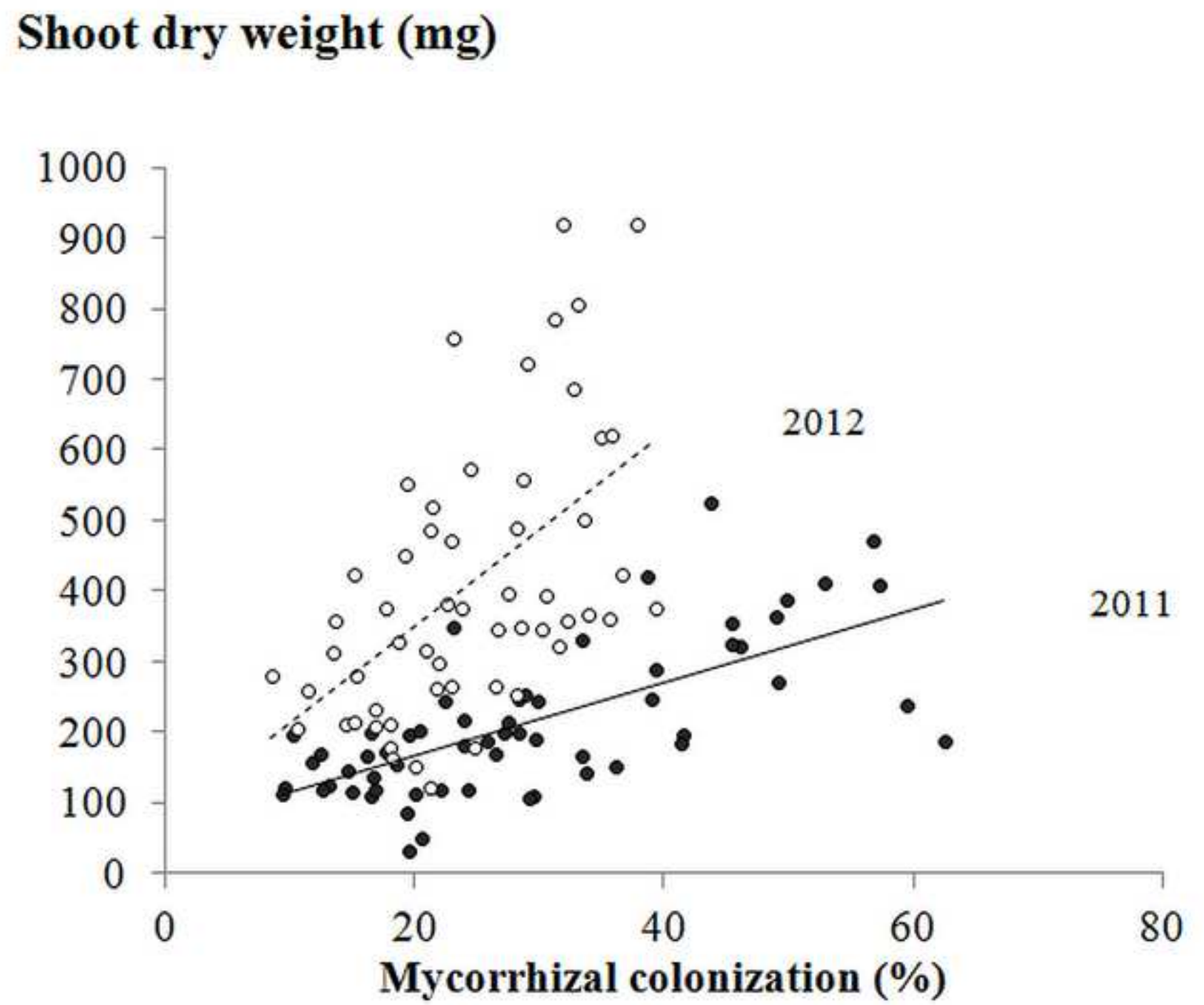

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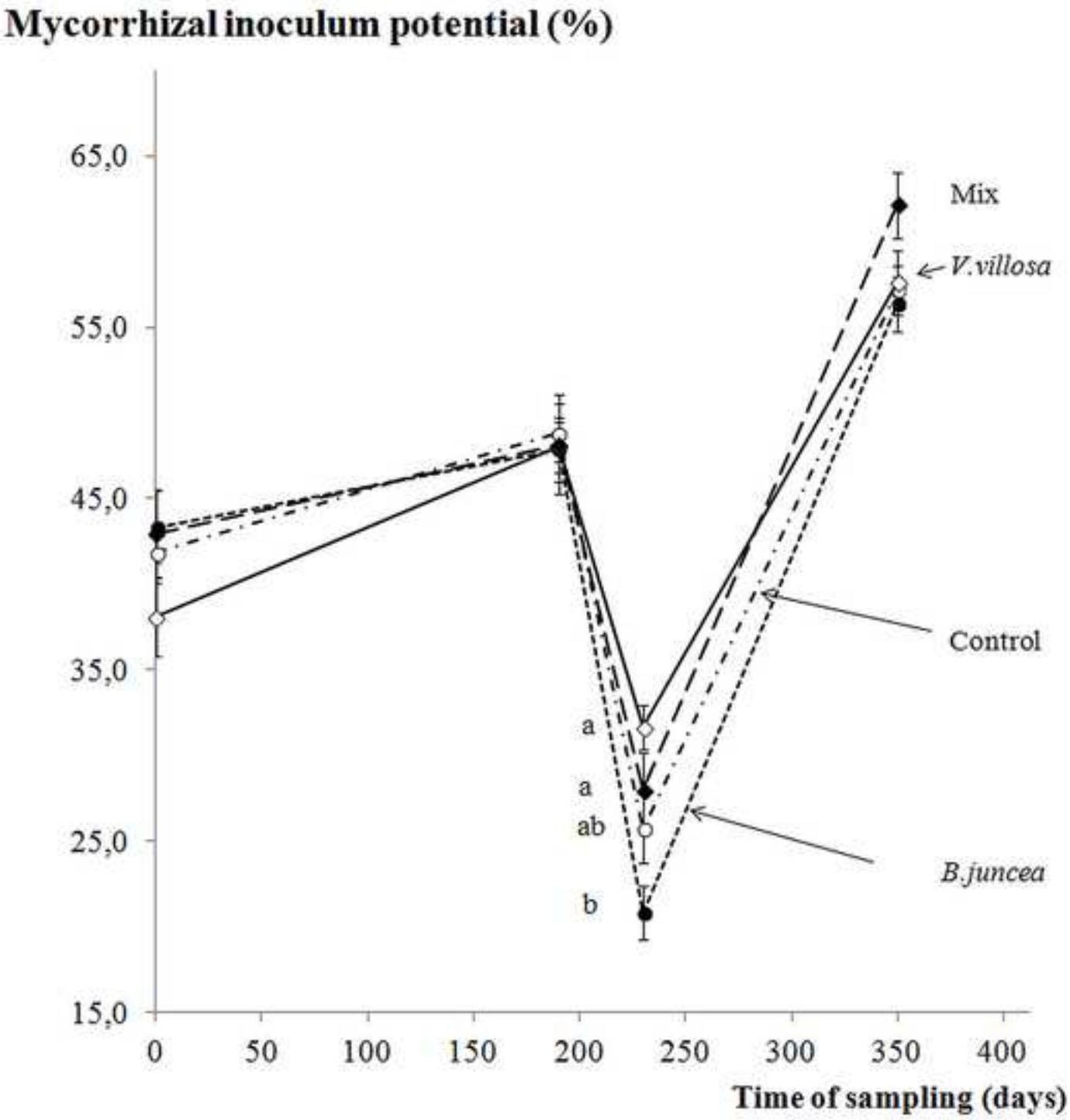

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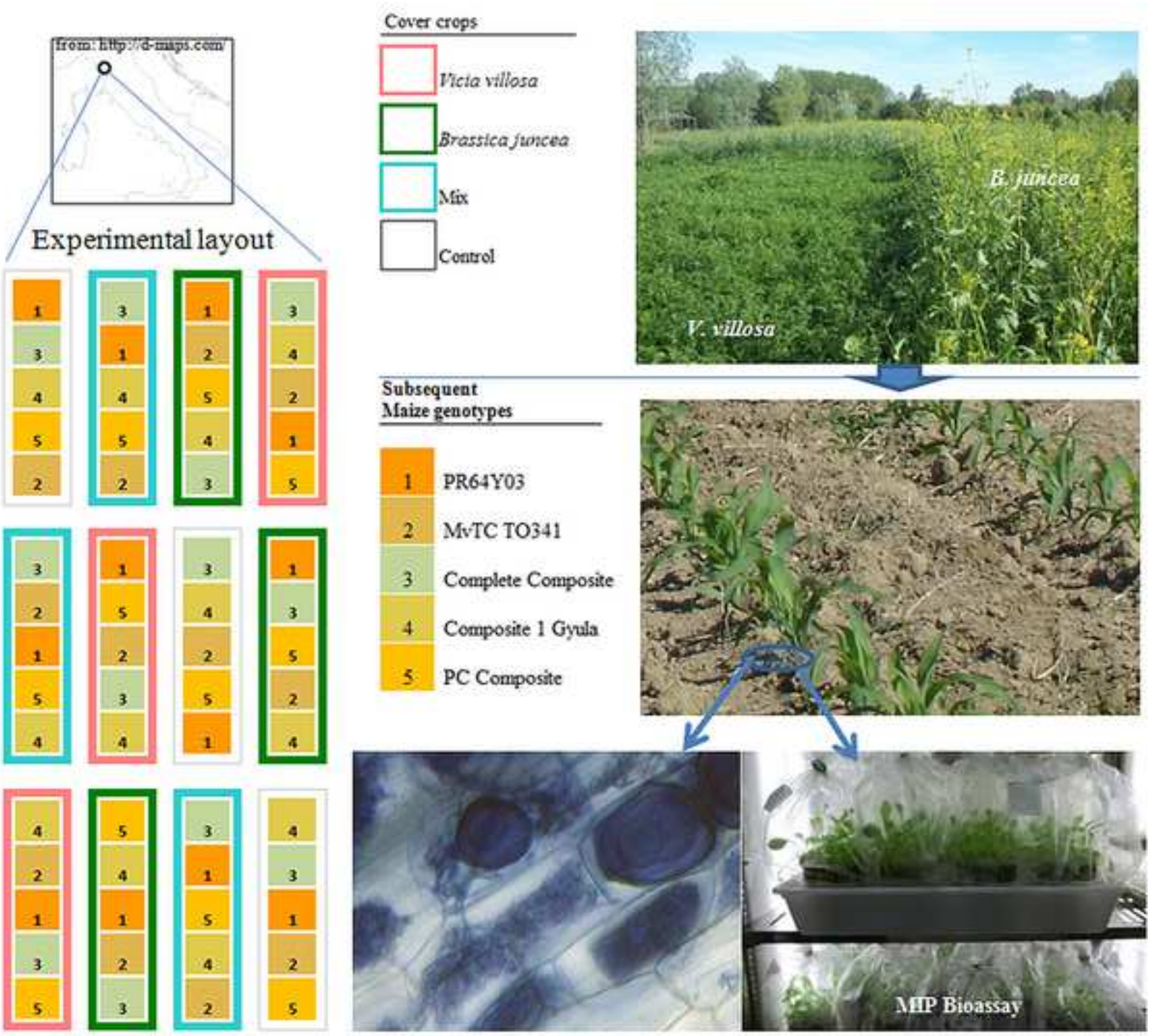

Figure 2
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