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

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1 **First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize**
2 **growth**
3

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13 **Abstract**
14 Arbuscular mycorrhizal fungi are increasingly used in organic cropping systems to increase yields.
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17 colonization in subsequent organic maize cultivars differing in the level of genetic diversity. Experiments
18 were conducted from 2010 to 2012 in a Mediterranean environment. First Indian mustard (*Brassica juncea*
19 L. Czern.), hairy vetch (*Vicia villosa* Roth), a mix of seven cover crop species (Mix) and natural vegetation
20 (Control) were cultivated as winter cover crops. Then an organically and a conventionally bred maize
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29 colonization of the subsequent crop and of soil mycorrhizal activity.
30

31 **Keywords** Cover crops – Organic agriculture – Arbuscular mycorrhizal fungi – Maize genotypes – Crop
32 diversity – Mycorrhizal inoculum potential
33
34

35 **1 Introduction**

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

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

52 Cover crops are widely recognized as an important management practice for sustainable agriculture
53 because of their contributions to soil conservation and quality, and to crop performance (Kabir and Koide
54 2002; Weil and Kremen 2007). They have been reported to help maintain or increase mycorrhizal potential
55 of soils, e. g. providing nourishment during winter periods to AMF, which are obligate mutualists (Kabir
56 and Koide 2002). When the agricultural fields lie fallow through the winter season, AMF populations are
57 deprived of carbohydrates, and consequently are considerably reduced by the start of the next cropping
58 season. Thus, mycotrophic cover crops may be fundamental in maintaining a high inoculum potential in the
59 absence of the cash crop during seasonal fallow periods.

60 Nonetheless, some cover crops - mainly members of the Brassicaceae family - are not mycorrhizal,
61 and may reduce AMF colonization in the subsequent crop. Some studies have indicated reduced
62 mycorrhizal colonization of the subsequent crop after the growth of a *Brassica* species (Gavito and Miller
63 1998; Koide and Peoples 2012) while others did not report any change (Pellerin et al. 2007; White and
64 Weil 2010). Thus, to delineate how cover crops influence field AMF populations it would be necessary to
65 have comparative field experiments that encompass both AMF host and non host cover crops.

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

69 crops (Tawaraya 2003; An et al. 2010). Plant breeding to create novel genotypes more efficient in nutrient
70 and water resource use represents a key target for sustainable agriculture. Crop breeding is generally
71 carried out in research stations where nutrients are not a limiting factor, possibly leading to the production
72 of hybrids less responsive to AMF. By contrast, breeding programs in organic agriculture should focus on
73 crop genotypes that make sustainable use of the available soil bioresources (Wolfe et al. 2008). Thus, a
74 profitable use of AMF in an organic and low-input farming context, will require the selection of a suitable
75 combination of plant host, fungal partner and agricultural management practices (Sawers et al. 2008).
76 Here, we tested the hypothesis that increasing the genetic (breeding) and species (cover crop)
77 diversity will provide a more favorable environment for AMF activity in an organic system (Fig. 1). The
78 specific aims of this study were: i) to assess the effects of three winter cover crop treatments, differing in
79 species diversity, and fallow on AMF colonization of five subsequent maize crop genotypes at the juvenile
80 stage and at harvest; ii) to monitor the effects of three winter cover crop treatments and fallow on soil
81 mycorrhizal potential; iii) to examine the growth responses of maize plants at juvenile stage and their
82 relationship with early mycorrhizal colonization; iv) to assess AMF susceptibility of two maize hybrids
83 (organically and conventionally bred) compared with three composite cross populations (organically bred)
84 of higher genetic diversity, at the juvenile stage and at harvest.
85
86 **2 Materials and methods**
87 2.1 Experimental site
88 The experimental fields were located at the Interdepartmental Centre for Agri-environmental Research
89 “Enrico Avanzi” (CIRAA) of the University of Pisa, located at S. Piero a Grado, Pisa (latitude 43°40’ N,
90 longitude 10°18’ E) in Italy. The fields are part of a long-term experimental system, MASCOT
91 (Mediterranean Arable Systems Comparison Trial) established in autumn 2001, comparing organic and
92 conventional management systems for a 5-year stockless arable crop rotation (Mazzoncini et al. 2010).
93 Physical and chemical characteristics of soil are: clay, 19.4%; silt, 29.2%; sand, 51.4%; pH (water) 8.3,
94 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
95 rotation includes maize (*Zea mays* L.), common wheat (*Triticum aestivum* L.), sunflower (*Helianthus*
96 *annuus* L.), pigeon bean (*Vicia faba* L. var. *minor*) and durum wheat (*Triticum durum* Desf.). The
97 experiment embeds additional organically-managed fields (‘organic playgrounds’) where specific plot
98 experiments are allocated (Bàrberi and Mazzoncini 2006).
99
100 2.2 Experimental design
101 The experiment was laid out in one organic playground as a split plot design with three blocks, and in each
102 year it was performed in a different field. Main plots included four soil cover treatments, namely *Brassica*

103 *juncea* (L.) Czern. cv. ISCI 20 (Indian mustard), *Vicia villosa* Roth cv. Latigo (hairy vetch), a mix of seven
104 species (hereafter called 'Mix') and a no-till fallow with natural vegetation (hereafter called 'Control'). The
105 Mix treatment, supplied as a commercial mixture by Arcoiris s.r.l. (Modena, Italy), included: *Fagopyrum*
106 *esculentum* Moench (buckwheat), *Lupinus albus* L. (white lupin), *Phacelia tanacetifolia* Benth. (lacy
107 phacelia), *Pisum sativum* L. (common pea), *Trifolium alexandrinum* L. (berseem clover), *Trifolium*
108 *incarnatum* L. (crimson clover) and *V. villosa.* Subplots included five maize genotypes, two hybrids
109 (Pioneer® PR64Y03 and MvTC TO341, developed under conventional and organic management
110 respectively) and three composite cross populations, namely Complete Composite, Composite 1 Gyula and
111 PC Composite. Composite cross populations are populations of segregating individuals formed by inter-
112 crossing seed stocks with divergent evolutionary origins, followed by bulking and propagation of the F1
113 progenies in successive cropping seasons (Phillips and Wolfe 2005). Compared to hybrids, they are thus
114 characterised by higher genetic diversity. Composite cross populations and the organic hybrid seeds were
115 provided by the Centre for Agricultural Research, Agricultural Institute, Hungarian Academy of Sciences,
116 Martonvásár. The whole trial was then composed of 60 subplots each measuring 3 × 10 m.
117
118 2.3 Cover crop management
119 Cover crops were sown on 18 October 2010 at a seeding rate of 9 kg ha^{-1} (*B. juncea)*, 100 kg ha^{-1} (*V.*
120 *villosa*) and 50 kg ha^{-1} (Mix). In 2011, cover crops were sown on 19 October at higher rates, since cover
121 crop biomass in the previous year was lower than expected and to ensure adequate plant stand: 12 kg ha^{-1}
122 (*B. juncea*), 120 kg ha^{-1} (*V. villosa*), and 65 kg ha^{-1} (Mix). Weeds were not controlled in any of the
123 treatments. Cover crops and weeds were sampled on 21 April 2011 and 23 April 2012 from four randomly
124 selected 0.25 m^2 quadrates plot^{-1}. Cover crop and weeds were separated and oven dried at 80°C until
125 constant weight. Total shoot dry biomass (cover crop and weeds) ranged from 165 g m^{-2} in Control to 200
126 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*,
127 900 in Mix and 920 in Control in 2012, weeds representing about 20-60% and 40-70% of the total biomass
128 in 2011 and 2012, respectively. In particular, in *B. juncea* weeds represented 64% and 47% of the total
129 biomass. The dominant weeds were represented by the AMF hosts *Lolium* spp., *Cynodon dactylon* (L.)
130 Pers. and *Avena* spp., which occurred as natural vegetation in Control treatment. No differences in weeds
131 distribution were observed among treatments. Each year, cover crops were mown at the end of April and
132 immediately incorporated into the soil by disc harrowing at a depth of 15 cm.
133
134 2.4 Maize sowing and management
135 Maize genotypes were sown on 26 April 2011 and 5 June 2012 at a spacing of 50 × 28 cm. Delayed sowing
136 in 2012 was due to prolonged heavy rain and cold. Nutex Letame (Sipcam Italia S.p.A., Pero, Italy), a

137 pelleted mixture of selected manures (NPK=3:3:3), was applied only in 2011 at 1000 kg ha^{-1} rate as a
138 starter fertiliser. Maize was grown as a rainfed crop, but in 2012 overhead irrigation was applied since an
139 extremely dry and hot period occurred after the juvenile stage.
140
141 2.5 Plant sampling
142 Maize plants were sampled for AMF root colonization at the 4th leaf (juvenile) phenological stage, and at
143 final harvest stage. At juvenile stage (16 May 2011 and 2 July 2012) the sampling was done by uprooting 4
144 plants from each subplot, to recover the whole root system. The plants were placed in polythene bags and
145 transported to the laboratory for analyses. Roots were processed for AMF assessment and shoots were oven
146 dried at 60°C for 5 days, then weighed and preserved in sealed bags for N and P analyses. At harvest stage,
147 4 soil cores measuring about 8 cm in diameter and 15 cm in depth were obtained from the base of the
148 sampled maize plants. The soil was washed through a 500 μm sieve to recover the roots.
149
150 2.6 Mycorrhizal root colonization of maize
151 At juvenile stage, maize roots were cleaned with tap water, cleared with 10% KOH in water bath at 80°C
152 for 15 min, neutralized in 2% aqueous HCl and stained with 0.05% trypan blue in lactic acid. Root
153 colonization was assessed under a dissecting microscope (Wild, Leica, Milano, Italy) at 25× or 40×
154 magnification by the gridline intersect method (Giovannetti and Mosse 1980).
155
156 2.7 Mycorrhizal inoculum potential of the experimental field soil
157 Mycorrhizal inoculum potential (MIP) bioassay before sowing was carried out to verify the homogeneity of
158 AMF propagules' distribution in the field soil. As *B. juncea* treatment reduced early AMF colonization in
159 the subsequent maize crop, in the second year we decided to assess MIP on soil samples at different times,
160 in order to investigate field AMF propagule density dynamics. Samples were taken: before cover crop
161 sowing; at the end of cover crop cycle, a few days before soil incorporation; after soil incorporation of
162 cover crops and tillage; at maize harvest. Soil samples (3 soil cores per subplot, taken 2.5 m apart at a depth
163 of 5 to 15 cm) were dried, sieved using a 4 mm sieve and put in 50 ml tubes. Three replicated tubes were
164 prepared for each MIP determination, for a total of 180 tubes. *Cichorium intybus* L. cv. Zuccherina di
165 Trieste was sown in tubes put in transparent sun bags and maintained in a growth chamber at 27 °C and
166 16/8 h light/dark daily cycle until harvest. One week after germination plants were thinned to four per tube.
167 Each tube was watered as needed. Plants were harvested 30 days after sowing and shoots excised and
168 discarded. After removing the soil from tubes, roots were separated and cleaned with tap water. Roots were
169 then cleared, stained and examined for AMF colonization assessment as described above.
170

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

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

217 Maize genotypes did not significantly influence AMF colonization at juvenile stage in both years:
218 all maize genotypes (both hybrids and composite cross populations) had a similar percentage of colonised
219 root length (25.1 to 28.8%), suggesting that at juvenile stage soil mycorrhizal potential may play a more
220 important role than genotype. Our results refer to the colonization of roots growing in the top soil layer (0-
221 15 cm), since root colonization and propagules numbers decrease with depth (>20 cm) (Oehl et al. 2005).

222

223 3.2 Dynamics of soil mycorrhizal inoculum potential

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

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

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

243 At maize harvest, MIP values were higher than values after cover crop soil incorporation ($F_{1,104}$ =
244 583.2; p <0.001), due to a generalized increase, which varied depending on the cover crop treatment ($F_{3,6}$ =
245 6.14; p= 0.03 for cover crop treatment; $F_{3,104}$ = 3.15; p= 0.028 for time × cover crop interaction) (Fig. 4).
246 Such a finding could be ascribed either to the growth of the host crop maize or to the favorable growing
247 season (spring-summer, compared with fall-winter) promoting soil microbial biomass, AMF spore
248 germination and spread of mycorrhizal networks in the soil (Gavito et al. 2002; Giovannetti et al. 2004).

249

250 3.3 Maize growth, N and P uptake at juvenile stage

251 Maize shoot dry matter at juvenile stage was significantly influenced by preceding cover crop ($F_{3,6}$=20.21,
252 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
253 only affected by genotype ($F_{4,30}$= 2.84, p = 0.041). In 2011 both shoot N and P contents were significantly
254 affected by cover crop treatments (p = 0.001 and 0.005 respectively) and genotypes (p = 0.014 and 0.017
255 respectively), while the interaction between the two was not significant (Table 1). Although cover crop
256 effect was only statistically significant in 2011, its effect on maize shoot biomass, N and P uptake followed
257 the same pattern in 2012: *V. villosa* > Mix > Control = *B. juncea* (Table 1), suggesting that *V. villosa* is a
258 good winter cover crop for the subsequent summer crop, when used as green manure, representing a source
259 of easily mineralisable N (Campiglia et al. 2010). In addition, *V. villosa*, as a N_2-fixing legume, can
260 accumulate a large amount of N during the growing period, and make it available to the subsequent crop.
261 The Mix treatment, containing species other than legumes, represents a less effective source of N than *V.*
262 *villosa*. Therefore a better AMF colonization may have contributed to the uptake of the additional N
263 available in soil (Hodge and Fitter 2010).

264 For each experimental year, we found a linear correlation between AMF root colonization and
265 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
266 2012, respectively) (Fig. 3). Maize, being a relatively short-season crop, is known to benefit from an early
267 and extensive mycorrhizal colonization both for juvenile growth and for grain yield at harvest (Bittman et
268 al. 2006), as confirmed in our experiment where grain yield was higher in those cover crop treatments (*V.*
269 *villosa* and Mix) which provide a higher early colonization level (N. Nol, personal communication).

270

271 3.4 Maize mycorrhizal colonization at harvest

272 At maize harvest, no significant differences in AMF root colonization among cover crop treatments were
273 detected, consistently with earlier studies reporting that the reduced AMF colonization of maize after
274 oilseed rape at the juvenile stage disappeared at silking (Gavito and Miller 1998). By contrast, percentage
275 of mycorrhizal colonization was significantly affected by genotypes ($F_{4,64}$=2.67, p = 0.040), while no effect
276 of cover crop×genotype interaction was found. Both maize hybrids showed a significantly lower AMF
277 colonization (29.2-30.0%), than composite cross populations (32.8-33.1%) in both years, as revealed by
278 orthogonal contrasts (p=0.002). However, the levels of colonization were high in both genotypes,
279 confirming that modern hybrids do not necessarily show low levels of colonization (An et al. 2010).
280

281 **4 Conclusions**

282 Our experimental findings show that cover crops management affects soil mycorrhizal potential
283 and early mycorrhizal colonization and growth of the subsequent maize crop. They also point out that
284 choice of the right (i.e. most AMF supportive or less detrimental for AMF) cover crop species is more
285 important than cover crop diversity (i.e. species mixture) in organic systems. Level of maize genetic
286 diversity did not seem to influence AMF symbiosis to a great extent. In addition, the monitoring of AMF
287 propagule dynamics over time evidenced that soil mycorrhizal potential values were negatively affected by
288 soil incorporation of cover crops. Further investigations will elucidate whether the strong negative impact
289 of *B. juncea* cover crop on AMF, reduced here by higher weed abundance under organic management, may
290 be additionally alleviated by avoiding tillage and soil incorporation of Indian mustard biomass which could
291 reduce the possible negative effects of isothiocyanates.
292

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301 **References**

302 An GH, Kobayashi S, Enoki H, Sonobe K, Muraki M, Karasawa T, Ezawa T (2010) How does arbuscular
303 mycorrhizal colonization vary with host plant genotype? An example based on maize (*Zea mays*)
304 germplasms. Plant Soil 327:441-453. doi:10.1007/s11104-009-0073-3

305 Avio L, Pellegrino E, Bonari E, Giovannetti M (2006) Functional diversity of arbuscular mycorrhizal
306 fungal isolates in relation to extraradical mycelial networks. New Phytol 172:347-357.
307 doi:10.1111/j.1469-8137.2006.01839.x
308 Bàrberi P, Mazzoncini M (2006) The MASCOT (Mediterranean Arable Systems Comparison Trial) long-
309 term experiment (Pisa, Italy). In: Raupp J, Pekrun C, Oltmanns M, Köpke U (eds) Long-term field
310 experiments in organic farming. ISOFAR Scientific Series No 1 Verlag Dr. Köster, Berlin, pp 1-14
311 Bittman S, Kowalenko CG, Hunt DE, Forge TA, Wu X (2006) Starter phosphorus and broadcast nutrients
312 on corn with contrasting colonization by mycorrhizae. Agron J 98:394-401.
313 doi:10.2134/agronj2005.0093
314 Campiglia E, Caporali F, Radicetti E, Mancinelli R (2010) Hairy vetch (*Vicia villosa* Roth.) cover crop
315 residue management for improving weed control and yield in no-tillage tomato (*Lycopersicon*
316 *esculentum* Mill.) production. Eur J Agron 33:94-102. doi:10.1016/j.eja.2010.04.001
317 Costanzo A, Bàrberi P (2013) Functional agrobiodiversity and agroecosystem services in sustainable wheat
318 production. A review. Agron Sust Dev 1-22. doi: 10.1007/s13593-013-0178-1
319 Fortuna P, Avio L, Morini S, Giovannetti M (2012) Fungal biomass production in response to elevated
320 atmospheric CO_2 in a *Glomus mosseae–Prunus cerasifera* model system. Mycol Progress 11:17-26.
321 doi:10.1007/s11557-010-0721-2
322 Galvez L, Douds DD, Wagoner P, Longnecker LR, Drinkwater LE, Janke RR (1995) An overwintering
323 cover crop increases inoculum of VAM fungi in agricultural soil. Am J Altern Agric 10:152-156.
324 Gavito ME, Miller MH (1998) Changes in mycorrhiza development in maize induced by crop management
325 practices. Plant Soil 198:185-192. doi:10.1023/a:1004314406653
326 Gavito ME, Bruhn D, Jakobsen I (2002) Phosphorus uptake by arbuscular mycorrhizal hyphae does not
327 increase when the host plant grows under atmospheric CO_2 enrichment. New Phytol 154:751-760.
328 doi:10.1046/j.1469-8137.2002.00404.x
329 Gianinazzi S, Gollotte A, Binet M-N, Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of
330 arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519-530. doi:10.1007/s00572-010-
331 0333-3
332 Giovannetti M, Avio L, Barale R, Ceccarelli N, Cristofani R, Iezzi A, Mignolli F, Picciarelli P, Pinto B,
333 Reali D, Sbrana C, Scarpato R (2012) Nutraceutical value and safety of tomato fruits produced by
334 mycorrhizal plants. Br J Nutr 107:242-251. doi:10.1017/S000711451100290X
335 Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular
336 mycorrhizal infection in roots. New Phytol 84:489-500
337 Giovannetti M, Sbrana C, Avio L, Strani P (2004) Patterns of below-ground plant interconnections
338 established by means of arbuscular mycorrhizal networks. New Phytol 164:175-181.

339 Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic
340 material has implications for N cycling. Proc Natl Acad Sci USA 107:13754–13759
341 Kabir Z (2005) Tillage or no-tillage: Impact on mycorrhizae. Can J Plant Science 85:23-29
342 Kabir Z, Koide RT (2002) Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient
343 uptake and yield of sweet corn in Pennsylvania, USA. Plant Soil 238:205-215.
344 Koide RT, Peoples MS (2012) On the nature of temporary yield loss in maize following canola. Plant Soil
345 360:259-269. doi:10.1007/s11104-012-1237-0
346 Mazzoncini M, Canali S, Giovannetti M, Castagnoli M, Tittarelli F, Antichi D, Nannelli R, Cristani C,
347 Bàrberi P (2010) Comparison of organic and conventional stockless arable systems: A
348 multidisciplinary approach to soil quality evaluation. Appl Soil Ecol 44:124-132.
349 Oehl F, Sieverding E, Ineichen K, Ris E-A, Boller T, Wiemken A (2005) Community structure of
350 arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed
351 agroecosystems. New Phytol 165:273–283. doi: 10.1111/j.1469-8137.2004.01235.x
352 Pellerin S, Mollier A, Morel C, Plenchette C (2007) Effect of incorporation of *Brassica napus* L. residues
353 in soils on mycorrhizal fungus colonisation of roots and phosphorus uptake by maize (*Zea mays* L.).
354 Eur J Agron 26:113-120. doi:10.1016/j.eja.2006.07.007
355 Phillips SL, Wolfe MS (2005) Evolutionary plant breeding for low input systems. J Agric Sci 143:245-254.
356 Pimentel D, Wilson C, McCullum C, Huang R, Dwen P, Flack J, Tran Q, Saltman T, Cliff B (1997)
357 Economic and environmental benefits of biodiversity. BioScience 47:747-757
358 Rinaudo V, Bàrberi P, Giovannetti M, van der Heijden MGA (2010) Mycorrhizal fungi suppress aggressive
359 agricultural weeds. Plant Soil 333:7-20. doi:10.1007/s11104-009-0202-z
360 Sawers RJH, Gutjahr C, Paszkowski U (2008) Cereal mycorrhiza: an ancient symbiosis in modern
361 agriculture. Trends Plant Sci 13:93-97. doi:10.1016/j.tplants.2007.11.006
362 Smith SE, Read DJ (2008) Mycorrhizal Symbioses, 3rd edn. Academin Press, New York
363 Tawaraya K (2003) Arbuscular mycorrhizal dependency of different plant species and cultivars. Soil Sci
364 Plant Nutr 49:655-668. doi:10.1080/00380768.2003.10410323
365 Weil R, Kremen A (2007) Thinking across and beyond disciplines to make cover crops pay. J Sci Food
366 Agric 87:551-557. doi:10.1002/jsfa.2742
367 White CM, Weil RR (2010) Forage radish and cereal rye cover crop effects on mycorrhizal fungus
368 colonization of maize roots. Plant Soil 328:507-521. doi:10.1007/s11104-009-0131-x
369 Wolfe MS, Baresel JP, Desclaux D, Goldringer I, Hoad S, Kovacs G, Löschenberger F, Miedaner T,
370 Østergård H, Lammerts van Bueren ET (2008) Developments in breeding cereals for organic
371 agriculture. Euphytica 163:323-346. doi:10.1007/s10681-008-9690-9
372

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
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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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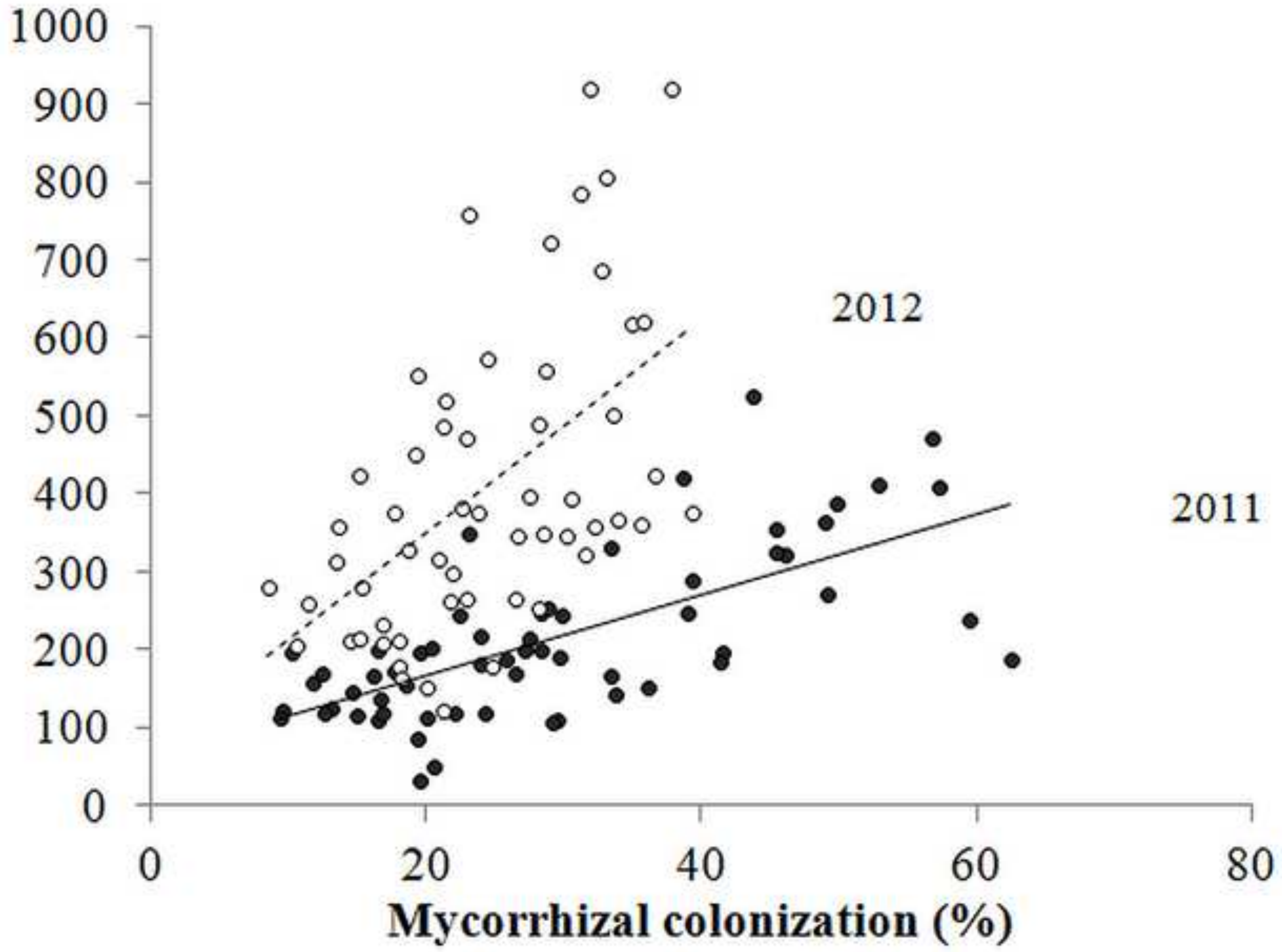

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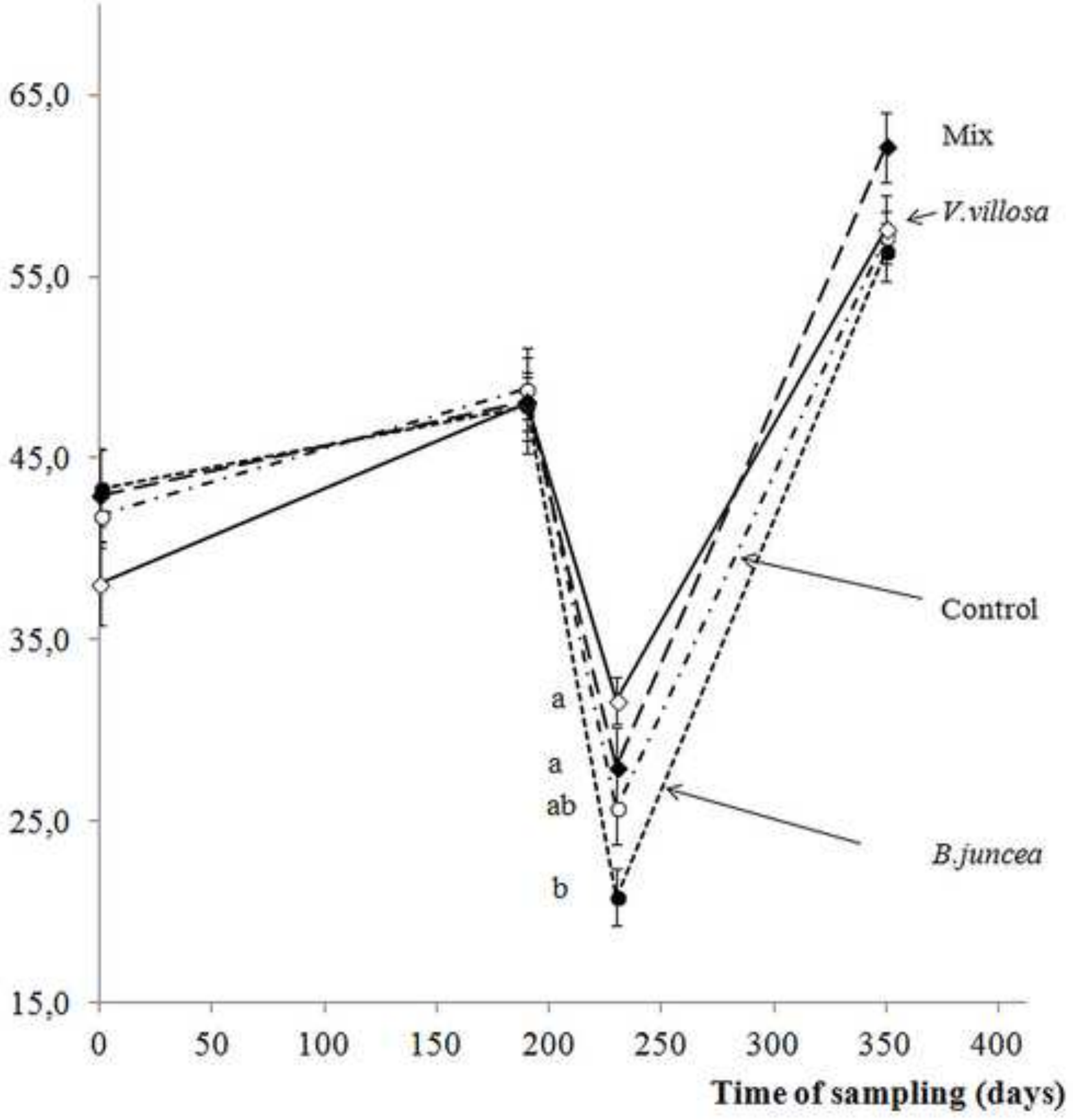

Figure 1

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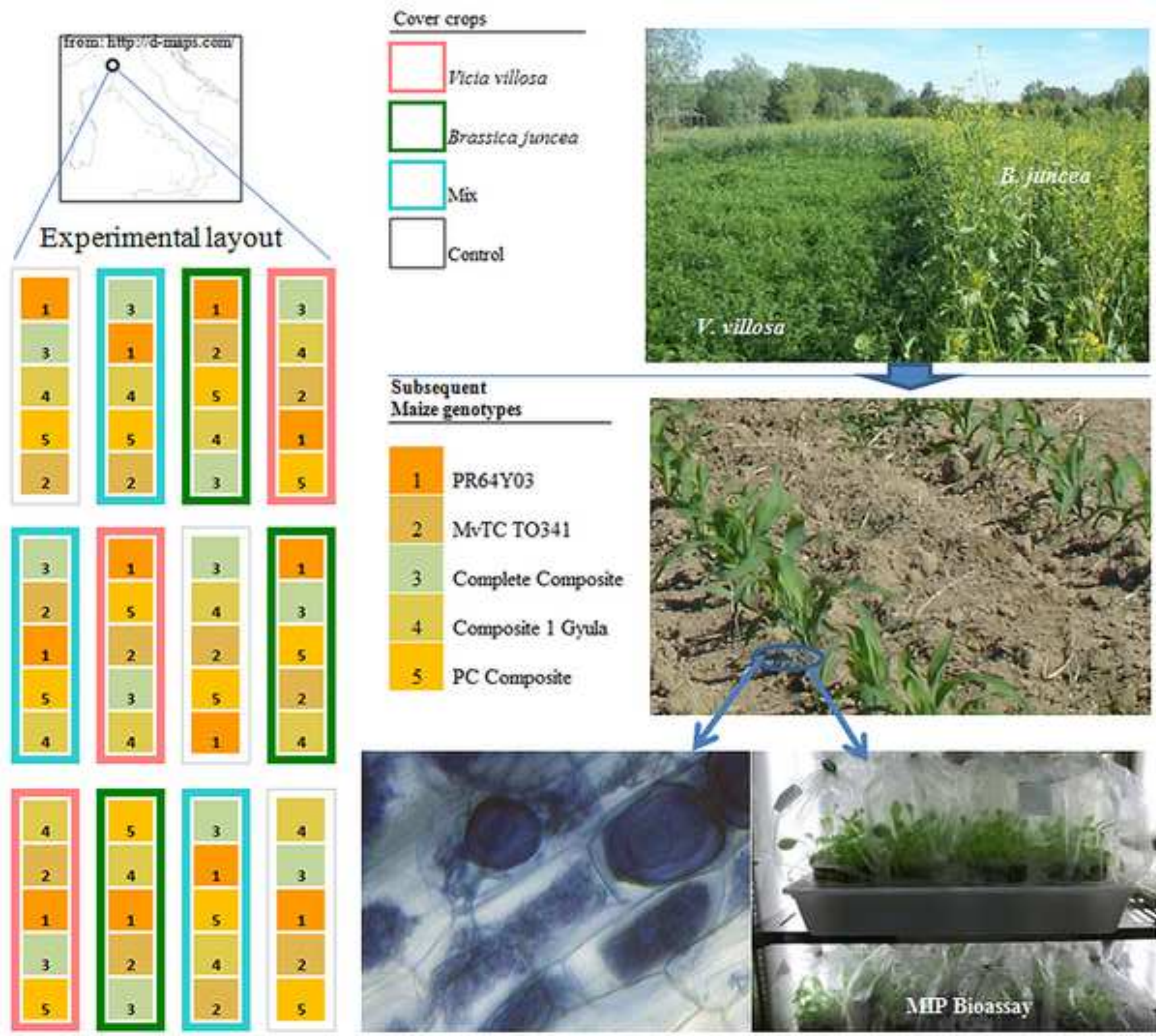

Figure 2
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