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(Article begins on next page)

1 **Effect of zeolite addition on soil properties and plant establishment**
2 **during forest restoration**

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13 **Abstract**

14 Zeolites, rocks with a content of zeolite group minerals greater than 50%, can
15 serve as support for plant nutrient uptake, by enabling the slow release of
16 macro- and micronutrients over time. Zeolites equally improve soil cation
17 exchange and water holding capacity. We analyzed the effect of zeolite
18 addition to the soil in forest restoration. We hypothesized that zeolite
19 ameliorated soil quality and consequently improved sward restoration and
20 seedling establishment.

21 Soil treatments were applied in 2014 and consist of: ploughed and ripped soil
22 (non-amended) and ploughed, ripped and amended with zeolite soil
23 (amended).

24 After application of the soil treatments, a herbaceous mixture was hand sown.
25 Oak (*Quercus robur* L.), hawthorn (*Crataegus monogyna* Jacq.) and elm (*Ulmus*
26 *minor* Mill.) seedlings were planted twice in the area, in autumn 2014 and
27 autumn 2015. We monitored soil, sward and seedlings from 2015 to 2017.
28 Seedling survival, which was generally above 50%, was never affected by
29 zeolite addition. The effect on seedling growth was significant only for
30 hawthorn planted in 2015 that was approximately 5 cm taller in the amended
31 soil. Herbaceous vegetation was also slightly affected by zeolite addition. The
32 zeolite increased nitrogen (N) and phosphorus (P) Olsen in 2015, and
33 exchangeable potassium (K) during the whole study period. Other parameters,
34 such as year and growing season had significant effects on soil, sward and
35 seedling dynamics. Time since restoration, plant-plant interactions, mechanical
36 site preparation and meteorological conditions all together influenced the
37 restoration dynamics more than zeolite application.

38 **Key words:** site preparation; zeolite; facilitation; grassland restoration; oak-
39 hornbeam forest; seedling performances.

40 **1. Introduction**

41 The building of transport infrastructure, such as motorways and high-speed
42 railways, entails damage, destruction, fragmentation and isolation of habitat
43 (Andrews, 1990; Cuperus et al., 1999; Forman, 2000). Construction activities
44 have been shown to severely affect vegetation and soil properties. Soil over-
45 compaction by the movement of heavy machinery alters soil structure. Bulk
46 density increases while soil permeability to air and water as well as water
47 holding capacity are reduced (Czyż, 2004; Batey, 2009). In compacted soil,

48 seedlings and saplings are more susceptible to drought, as the root system is
49 prevented from quickly reaching deep and moist horizons (Bassett et al., 2005;
50 Sinnett et al., 2008; Self et al., 2012; Cambi et al., 2018). Topsoil loss, its mixing
51 with subsoil or construction waste during storage and replacement may reduce
52 soil quality (DEFRA, 2009). Vegetation and litter removal also contribute to the
53 deterioration of soil properties. A lack in the provision of organic matter
54 influences soil aggregate stability, soil porosity, gas exchange reactions and
55 water relations (Schoenholtz et al., 2000).

56 As soil physical and chemical properties directly drive the success of
57 revegetation in degraded areas, soil recovery should be a fundamental step in
58 restoration projects (Heneghan et al., 2008; Haan et al., 2012). Ploughing,
59 mounding and subsoiling can help to loosen soil while improving porosity, water
60 infiltration rate, and useful soil volume (Bocio et al., 2004; Löff et al., 2012). In
61 addition, the use of organic amendments, such as sawdust, sewage sludge,
62 bark mulch or compost, can contribute to the creation of more desirable planting
63 sites (Cogger, 2005; Sheoran, 2010). However, due to organic matter
64 decomposition, the action of organic amendments on soil nitrogen (N) and
65 carbon (C) availability for plants decreases over time (Sullivan et al., 2003).

66 In the restoration of degraded systems, the use of slow release amendments
67 that produce long-term effects on soil properties, such as zeolitites, should be
68 effective (Ming and Allen, 2001; Tan et al., 2018). Zeolitites are rocks with a
69 content of zeolites, hydrated alkaline aluminosilicates, greater than 50%. The
70 infinite, open, three-dimensional structure of zeolites, gives them the capacity to
71 retain a large amount of water (Xiubin and Zhanbin, 2001) and they also have a

72 high and selective cation exchange capacity (CEC). Zeolites uptake macro and
73 micronutrients such as potassium (K), calcium (Ca), sodium (Na), magnesium
74 (Mg) and nitrogen (N) (Polat et al., 2004; Ozbahce et al., 2015), retain beneficial
75 nutrients in the root zone and improve nutrients availability for plants.

76 Application of zeolites to the soil improves plant performances in crops (e.g.
77 Shahbaz et al., 2018), grassland restoration (e.g. Buondonno et al., 2013), and
78 orchards (e.g. Milosevic and Milosevic, 2009), but little is known about its
79 effects on other woody plants with uncertain results (Ayan and Tufekcioglu,
80 2006). During a restoration trial, we investigated whether zeolite can improve
81 seedlings survival and growth of pedunculate oak (*Quercus robur* L.), hawthorn
82 (*Crataegus monogyna* Jacq.) and elm (*Ulmus minor* Mill.). We hypothesized
83 that mechanical site preparation and zeolite addition may improve soil physical
84 and chemical properties, thereby ameliorating seedling establishment. We also
85 tested for zeolite effects on sward restoration as grassland vegetation can
86 affect seedling performances. In severe environments, such as construction
87 yards without vegetation cover, herbaceous species may provide shade and
88 help seedling establishment and survival (Liancourt et al., 2005; Wright et al.,
89 2014). Herbaceous vegetation can act as nurse plants for woody species, as it
90 can improve soil moisture, C and N content, and reduce soil temperature
91 (Holmgren et al., 1997; Maestre et al., 2001). Even if soil properties directly
92 influence tree and herbaceous vegetation dynamics, few restoration
93 experiments have considered the evolution of both over time (Franklin et al.,
94 2012).

95 In this trial, we used zeolite to accelerate secondary succession. We monitored
96 soil, herbaceous vegetation and seedling conditions over time, in order to study
97 the mechanisms driving short-term dynamics. We hypothesized that zeolite
98 addition to soil would: 1) improve tree and shrub seedling survival rate and
99 growth 2) accelerate sward restoration; 3) accelerate the recovery rates of soil
100 properties, particularly by increasing CEC.

101 **2. Materials and methods**

102 **2.1 Study site.**

103 The experiment was conducted in a 1.49 ha degraded area in north-western
104 Italy, on the Po Plain (45°11'38.60" N, 7°50'38.04" E, ca. 190 m a.s.l.). The site
105 (already described in Martelletti et al., 2018) was located along the Rail Freight
106 Corridor 6 (Mediterranean Corridor) (European Commission, 2018), linking the
107 southwestern Mediterranean region of Spain and the Hungarian border with
108 Ukraine, and it was surrounded by the A4 motorway. The climate was
109 temperate, with an annual mean temperature of 11.6 °C and average annual
110 precipitation of 806.9 mm (ARPA Piemonte – Verolengo meteorological station,
111 10 km from the study site, period 1991-2017). Rainfall was not homogeneously
112 distributed during the year, with spring and autumn being the wettest seasons.

113 The area was a road constructor's yard, during the building of the motorway.
114 Contrary to other yards located along the Corridor, where topsoil was stripped
115 away and stockpiled, here it was never removed. The area was used to stock
116 soil from other sites and inert waste. In November 2014, after the roadworks
117 ended, the area was restored. Using a tractor, the soil was ripped (to a 70 cm
118 depth) and ploughed (to a 40 cm depth) to reduce compaction caused by the

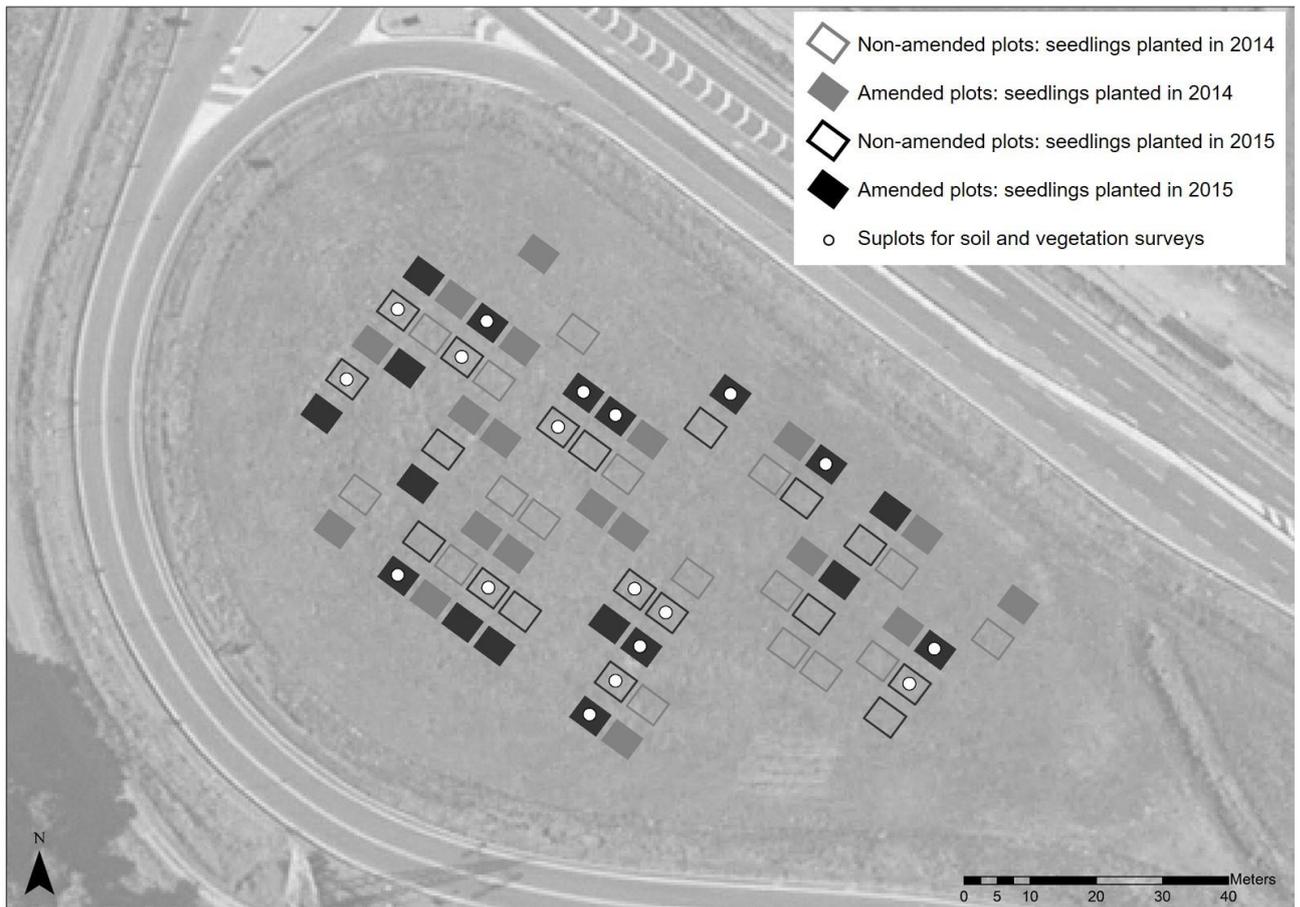
119 movement of heavy machinery and stockpiling. In the topsoil (10 cm depth) the
120 texture was sandy-loam. The pH was moderately alkaline (pH measured in H₂O
121 ranged from 7.4 to 8.0). Soil organic matter (SOM) content in the topsoil ranged
122 from 1.27% to 2.58% while the N content ranged between 0.07% and 0.15%.
123 The CEC ranged between 6.94 and 9.62 meq/100 g while P Olsen and K
124 ranged from 8.25 to 12.43 µg/g and from 0.20 to 0.80 meq/100 g, respectively.
125 No trees or herbaceous cover were present when the experiment started. Tree
126 and shrub seedlings (two-year-old containerized nursery plants) were therefore
127 planted twice, in November 2014 and November 2015, while a grassland
128 species mixture was hand sown in spring 2015. The seedlings used in the
129 experiment were typical species of the lowland oak-hornbeam Mesophytic
130 deciduous forest which represents the natural late seral forest ecosystem of the
131 Po Plain, dominated by *Q. robur* and *Carpinus betulus* L. The grassland species
132 mixture was composed of 10% *Lolium perenne* L. (perennial ryegrass), 40%
133 *Festuca gr. rubra* L. (red fescue), 15% *Poa pratensis* L. (Kentucky bluegrass),
134 25% *Lotus corniculatus* L. (bird's-foot trefoil), and 10% *Trifolium repens* L.
135 (white clover). Grassland species were chosen among those that can contrast
136 the presence of invasive herbs due to their competitiveness and longevity
137 (Barker, 1995).

138 At the beginning of the experiment, the area was fenced off against the
139 introduced eastern cottontail (*Sylvilagus floridanus* Allen) and other wild
140 herbivores (mainly *Capreolus capreolus* L. and *Sus scrofa* L.) to avoid browsing
141 damage to seedlings.

142 **2.2 Experimental design**

143 The experiment lasted three years, from November 2014 to October 2017. The
144 experimental design (Fig. 1) included two soil treatments (non-amended and
145 amended with zeolite, hereinafter amended). Zeolite accounts for about 70%
146 of the total mine material, which is mainly a kind of mordenite. The zeolite used
147 in the experiment was characterized by: 3-8 mm diameter; CEC 140 meq/100g;
148 exchangeable Ca 48.19 meq/100g; exchangeable Mg 4.32 meq/100g;
149 exchangeable K 25.16 meq/100g.

150 The site was divided into nine 4 m wide rows (2.5 m apart, approximately). After
151 the mechanical site preparation described above, the rows were alternatively
152 amended using 43 Mg/ha of zeolite, in order to raise the CEC to 15 meq/100g
153 (according to the technical guideline reported in Curtaz et al., 2013). Within the
154 nine rows, 72 rectangular plots (4 x 5 m wide) were established as reported in
155 Fig.1.



156

157 **Fig. 1.** Experimental design. Rows non-amended (no colour) alternated with
 158 those amended with zeolitite (fully coloured). The grey plots were planted in
 159 November 2014 and the black ones in November 2015. White circles indicate
 160 the plots in which we conducted soil and vegetation surveys. A motorway
 161 surrounded the study area.

162

163 Pedunculate oak (*Quercus robur* L.), hawthorn (*Crataegus monogyna*, Mill.),
 164 and elm (*Ulmus minor*, Mill.) seedlings used for the restoration of the area were
 165 planted 80 cm apart in 30 cm depth holes, using a spade. For both soil
 166 treatments, 30 seedlings per species were planted in a plot; with a total of six
 167 plots per species. The two-year-old containerized seedlings were from a

168 Regional nursery located 50 km away from the area (“Fenale” Regional nursery
169 – Albano Vercellese, 45°26'16.87" N, 8°23'17.85" E, ca. 154 m a.s.l.). Planting
170 was performed twice, in November 2014 (2014) and in November 2015 (2015),
171 thus allowing us to study the influence of different meteorological conditions on
172 seedlings performances during the first (First) and second (Second) growing
173 season (thus, for seedlings planted in 2014: first growing season from April to
174 October 2015 = First2014; second growing season from April to October 2016 =
175 Second2014. For seedlings planted in 2015: first growing season from April to
176 October 2016 = First2015 and second growing season from April to October
177 2017 = Second2015). In total 36 plots (2 soil treatments x 6 plots x 3 species)
178 were planted each year. At planting time, in November 2014, pedunculate oak,
179 hawthorn, and elm seedlings had a mean height of 61.4 cm (\pm 13.0), 57.3 cm (\pm
180 9.1) and 74.0 cm (\pm 8.0), respectively. In November 2015 mean heights were
181 33.3 cm (\pm 3.7), 50.5 cm (\pm 3.0) and 72.9 cm (\pm 20.9) respectively.

182 We also investigated the response to the addition of zeolite and the dynamics
183 over time of the grassland species mixture sown.

184 **2.3 Data collection**

185 **2.3.1 Ancillary measurements**

186 Air temperature and precipitation were recorded hourly by means of an
187 automatic meteorological station (Verolengo) located 10 km from the study site
188 (45°10'59.5" N, 8°00'37.9" E; 163 m a.s.l.) and belonging to ARPA Piemonte.
189 Soil temperature and moisture at 10 cm soil depth were recorded at 30-minute
190 intervals each summer (from the 1st of June to the 31st of August – J, J, A),
191 using Lascar EasyLog EL-USB-2 dataloggers and WatchDog A-Series Data

192 Logger with external temperature sensors for temperature and Spectrum
193 WatchDog 1400 Micro Stations with Spectrum WaterScout SM 100 soil
194 moisture sensors for moisture.

195 **2.3.2 Seedlings**

196 Seedlings survival and height were recorded twice a year, in May and
197 September 2015, 2016, and 2017. Seedlings were recorded as dead or alive,
198 and the survival rate was calculated within the plot. Height was measured with a
199 ruler on all the living plants as the stretched length from the ground to the
200 highest living sprout. Growth increments were calculated as the difference
201 between plant height in September and May of the same year.

202 **2.3.3 Herbaceous vegetation**

203 Herbaceous vegetation was surveyed once a year in late June. Surveys were
204 conducted in 18 sub-plots of 2 x 2 m (nine in the non-amended soil and nine in
205 the amended), centered within each rectangular plot. Herbaceous layer height
206 was measured as the mean of five randomized measurements, performed with
207 the sward stick method (Stewart et al., 2001). Total herbaceous species cover
208 was visually estimated, then species cover was recorded for all species with
209 >1% cover. A minimum value of 0.3% was assigned to occasional species
210 (Tasser and Tappeiner, 2005) so that a complete list of all plant species in the
211 sub-plot was recorded.

212 **2.3.4 Soil sampling and laboratory analysis**

213 After the initial mechanical preparation and zeolite addition, soil samples
214 (topsoil, 0-10 cm depth) were collected in the middle of all the 18 sub-plots
215 already used for vegetation surveys, once a year in late June. All samples were

216 dried, sieved, and analyzed for the determination of pH, CEC, electrical
217 conductivity (EC), exchangeable base content (Mg, K, Ca), total carbon (TC),
218 inorganic carbon (IC), organic carbon (OC), and total nitrogen (TN). pH and EC
219 were measured in water (soil:water = 1:2.5). The CEC was measured by the
220 barium chloride extraction (pH 8.1) method.. Exchangeable base content and
221 saturation, on the barium chloride extracts, were measured by AAS (flame
222 atomic absorption spectrometer, Analyst 400, Perkin Elmer, Waltham, MS,
223 USA). For the analysis of TC and TN, soil aliquots were milled and analyzed by
224 dry combustion with a CN elemental analyzer (CE Instruments NA2100,
225 Rodano, Italy). IC represented by the carbonate content was determined with a
226 Dietrich Fruhling pressure-calimeter. Total organic carbon (TOC) was
227 calculated from the difference between TC and IC. Bicarbonate extractable
228 phosphorus (P Olsen), assumed to represent readily bioavailable phosphate,
229 was determined according to Olsen et al. (1954) with P detection by molybdate
230 colorimetry (Murphy and Riley, 1962).

231 **2.4 Data analysis**

232 Differences among years in terms of summer (J, J, A) air temperature and
233 precipitation, and summer soil temperature and moisture were analyzed
234 performing one-way ANOVAs.

235 Assumptions of normality and homogeneity of variance were checked ($p \geq 0.05$)
236 with Shapiro-Wilk and Levene tests, respectively. Variables that were not
237 normally distributed were log-transformed prior to further statistical analysis.
238 When normal distribution or homogeneity of variances were not met, even after
239 log-transformation, the non-parametric Kruskal-Wallis test was used. When

240 significant differences were found, Tukey's and Mann-Whitney's *post hoc* tests
241 were performed for ANOVAs and Kruskal-Wallis tests, respectively.

242 Seedling performances (survival and growth rate) were compared between
243 years (2014 vs 2015), growing season (First vs Second), and soil treatments
244 (non-amended vs amended). Firstly, the role of zeolite was tested. As zeolite
245 addition never affected the number of survived plants, we did not further
246 consider soil treatment in survival rate analyses. Secondly, in order to test for
247 the effect of plantation time on seedling performances, we compared survival
248 and growth rate between growing seasons (First2014 vs Second2014 and
249 First2015 vs Second2015). The effect of planting year on survival and growth
250 rate was also assessed by testing First2014 vs First2015 and Second2014 vs
251 Second2015. Significance of differences was tested using Student's T-tests for
252 independent samples (or Mann-Whitney U tests, when normal distribution was
253 not met).

254

255 Grassland species diversity was assessed for each sub-plot by calculating
256 species richness (i.e. the total number of recorded species) and Shannon
257 diversity index (Magurran, 1988). Each plant species was associated to its
258 phytosociological optimum (at class level) according to Aeschimann et al.
259 (2004). This approach has been widely used to evaluate vegetation response to
260 management and disturbance regimes in different ecosystems (Lonati et al.,
261 2013; Moris et al., 2017; Orlandi et al., 2016; Pittarello et al., 2016; Vacchiano
262 et al., 2016). Therefore, according to their phytosociological optimum, plant
263 species were grouped into three vegetation units, following an increasing

264 gradient of habitat naturalness: i) annual ruderal species (i.e. species belonging
265 to *Stellarietea mediae* class), ii) perennial ruderal species (i.e. species
266 belonging to *Agropyretea intermedii-repentis* and *Artemisietea vulgaris* classes),
267 and iii) typical grassland species (i.e. species belonging to *Molinio-*
268 *Arrhenatheretea* class). The percentage cover of the following herbaceous
269 species assemblages was then calculated for each plot: 1) sown species; 2)
270 total spontaneous species (i.e. species not belonging to the sown mixture)
271 divided into annual and perennial (according to Landolt et al., 2010); 3) annual
272 and perennial ruderal spontaneous species; 4) typical grassland species; 5)
273 alien species (i.e. the cumulative cover of *Ambrosia artemisifolia* L., *Artemisia*
274 *verlotorum* Lamotte, *Erigeron annuus* (L.) Pers., *Helianthus tuberosus* L.,
275 *Lepidium virginicum* L., *Oxalis fontana* Bunge, *Solidago gigantea* Aiton., *Thlaspi*
276 *arvense* L., *Veronica persica* Poiret).

277 Herbaceous vegetation parameters were compared between soil treatments
278 (non-amended vs amended) using Student's T-test for independent samples (or
279 Mann-Whitney U tests, when normal distribution was not met).

280 Differences in soil physical parameters (temperature and moisture) and soil
281 chemistry (pH, EC, CEC, exchangeable base content, TC, IC, OC, and TN)
282 were also analyzed, applying the same statistical approach.

283 Soil temperature and moisture, herbaceous vegetation dynamics and soil
284 chemical parameters within the same treatment were compared among years,
285 using repeated measure ANOVAs (or Friedman's tests, when homogeneity of
286 variances was not met). When significant differences were found, Tukey's and

287 Wilcoxon's *post hoc* tests were performed for repeated measure ANOVAs and
288 Friedman's tests, respectively.

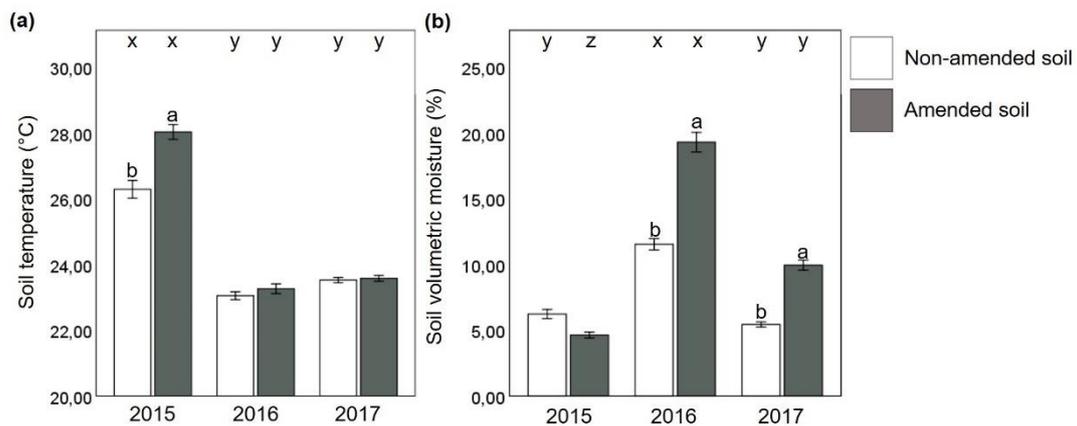
289 All tests were performed using PAST 3.20 (Hammer et al., 2001).

290 **3. Results**

291 **3.1 Meteorological and pedoclimatic conditions**

292 The summer season (J, J, A) was significantly cooler ($p < 0.05$) in 2016 than
293 2015 (mean air temperature of 22.1 °C vs 23.2 °C, respectively), while 2017 did
294 not differ ($p > 0.05$) from either of them (mean air temperature of 22.8 °C). The
295 cumulative rainfall increased over the years (168.2 mm in 2015, 190.2 mm in
296 2016 and 243.0 mm in 2017), without significant differences among years
297 ($p < 0.05$). All summers were warmer than the 1991-2017 period, in which a
298 mean air temperature of 21.4 °C was recorded. 2015 and 2016 were dryer and
299 2017 was wetter than the 1991-2017 period (mean cumulative rainfall of 197.0
300 mm).

301 During summer 2015 soil temperature reached higher values, in both non-
302 amended and amended treatments, than the subsequent summer seasons (Fig.
303 2a). In particular, the highest soil temperature was observed in amended plots.
304 The zeolite did not affect soil volumetric moisture during the first summer
305 season while in 2016 and 2017 the highest values were measured in the
306 amended treatment. Over time soil volumetric moisture reached the highest
307 values in the second summer (Fig. 2b).



308

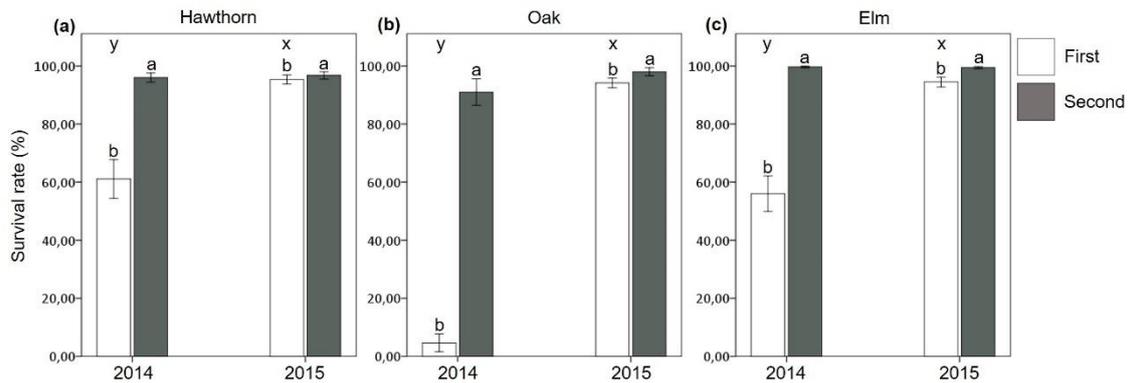
309 **Fig. 2.** Mean soil temperature (a) and volumetric moisture (b) during the three
 310 summer seasons (J, J, A). “a” and “b” indicate significant differences between
 311 treatments ($p < 0.05$). “x”, “y”, and “z” indicate significant differences among
 312 years for each treatment ($p < 0.05$). Letters are not shown when differences are
 313 not significant ($p \geq 0.05$). Data are presented as mean \pm standard error.

314

315 **3.2 Seedlings**

316 As reported above, zeolite addition never affected the survival rate. The
 317 number of living plants at the end of the first growing season was significantly
 318 lower than the survival rate at the end of the second growing season for both
 319 planting years, for all the species (Fig. 3). At the end of the first growing season,
 320 First2014 seedlings showed a lower survival rate than First2015 ones. No
 321 significant differences were detected for survival rate between the two planting
 322 years at the end of the second growing season (Second2014 vs Second2015)

323 (Fig.3 a, b, and c).



324

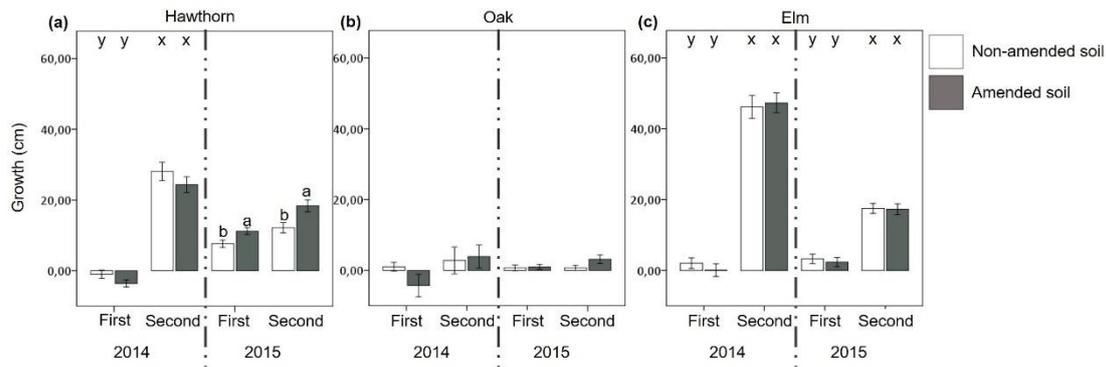
325

326 **Fig. 3.** Survival rate of (a) *C. monogyna* (hawthorn); (b) *Q. robur* (oak); (c) *U.*
327 *minor* (elm) for each planting year (2014 and 2015) and growing season (First
328 or Second). “a” and “b” indicate significant differences between first and second
329 growing season ($p < 0.05$). “x” and “y” indicate significant differences between
330 the same growing season for each planting year ($p < 0.05$). Letters are not
331 shown when differences are not significant ($p \geq 0.05$). Data are presented as
332 mean \pm standard error.

333

334 Soil treatments influenced the growth of hawthorn planted in 2015, with taller
335 seedlings in the amended than non-amended soil for both First2015 and
336 Second2015 (Fig. 4a). No other significant soil effects were observed on plant
337 growth (Fig. 4 b and c).

338 Growing season affected seedlings growth for hawthorn planted in 2014 and for
339 elm planted both in 2014 and 2015, in both soil treatments (Fig. 4 a and c,
340 respectively). No differences in oak between the two growing seasons were
341 recorded (Fig. 4b).



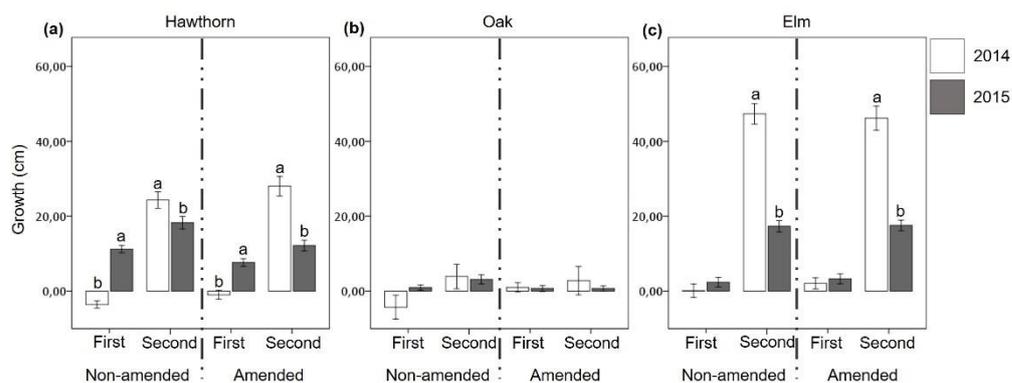
342

343 **Fig. 4.** Growth of (a) *C. monogyna* (hawthorn); (b) *Q. robur* (oak); (c) *U. minor*
 344 (elm) for the two planting years (2014 and 2015) and growing seasons (First
 345 and Second). “a” and “b” indicate significant differences between soil treatments
 346 ($p < 0.05$). “x” and “y” indicate significant differences between the first and
 347 second growing season for each soil treatment ($p < 0.05$). Letters are not shown
 348 when differences are not significant ($p \geq 0.05$). Data are presented as mean \pm
 349 standard error.

350

351 The year influenced hawthorn and elm growth (Fig. 5 a and c, respectively).
 352 When comparing the two first growing seasons of hawthorn seedlings, they
 353 showed a negative growth in First2014 significantly lower than First2015, for
 354 both soil treatments. Instead, both hawthorn and elm showed higher growths in
 355 Second2014 than in Second2015, independently of soil treatment. No year
 356 influence was observed on oak (Fig. 5b).

357



358

359 **Fig. 5.** Growth of (a) *C. monogyna* (hawthorn); (b) *Q. robur* (oak); (c) *U. minor*
 360 (elm) for the two soil treatments (non-amended and amended) and growing
 361 seasons (First or Second). “a” and “b” indicate significant differences between
 362 planting year for each growing season ($p < 0.05$). Letters are not shown when
 363 differences are not significant ($p \geq 0.05$). Data are presented as mean \pm standard
 364 error.

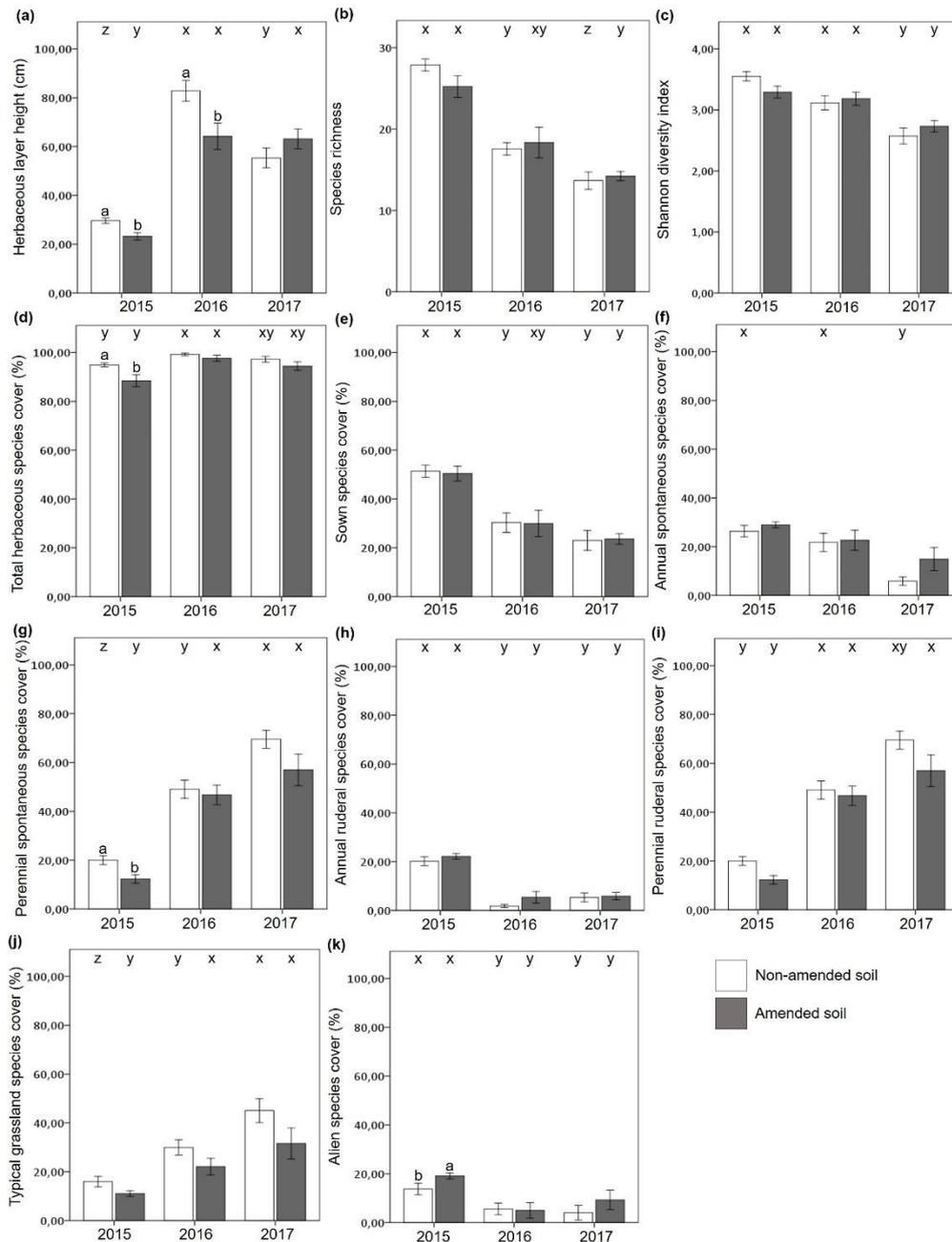
365

366 3.3 Herbaceous vegetation

367 Soil treatment significantly affected herbaceous species cover in 2015 (Fig. 6d),
 368 herbaceous layer height in 2015 and 2016 (Fig. 6a), and perennial spontaneous
 369 species cover in 2015 (Fig. 6g), with lower values in the amended soil. No other
 370 significant soil treatment effects were detected on herbaceous vegetation (Fig.
 371 6).

372 Year significantly affected all parameters, in both the non-amended and
 373 amended soil, except for annual spontaneous species cover in the amended
 374 soil (Fig. 6f). Species richness (Fig. 6b) and Shannon diversity index (Fig. 6c)
 375 decreased more from 2015 to 2017 in the non-amended than in the amended
 376 treatment. Herbaceous cover differed among years, with a progressive increase

377 of perennial (spontaneous and ruderal, Fig. 6 g and i) species and typical
 378 grassland species (Fig. 6j) cover while sown species (Fig. 6e) and annual
 379 (spontaneous and ruderal, Fig. 6 f and h) species cover decreased.



380

381 **Fig. 6.** Herbaceous cover dynamics in non-amended and amended treatments
 382 during the three study years. (a) Herbaceous layer height; (b) Species richness;
 383 (c) Shannon diversity index; (d) Herbaceous species cover; (e) Sown species

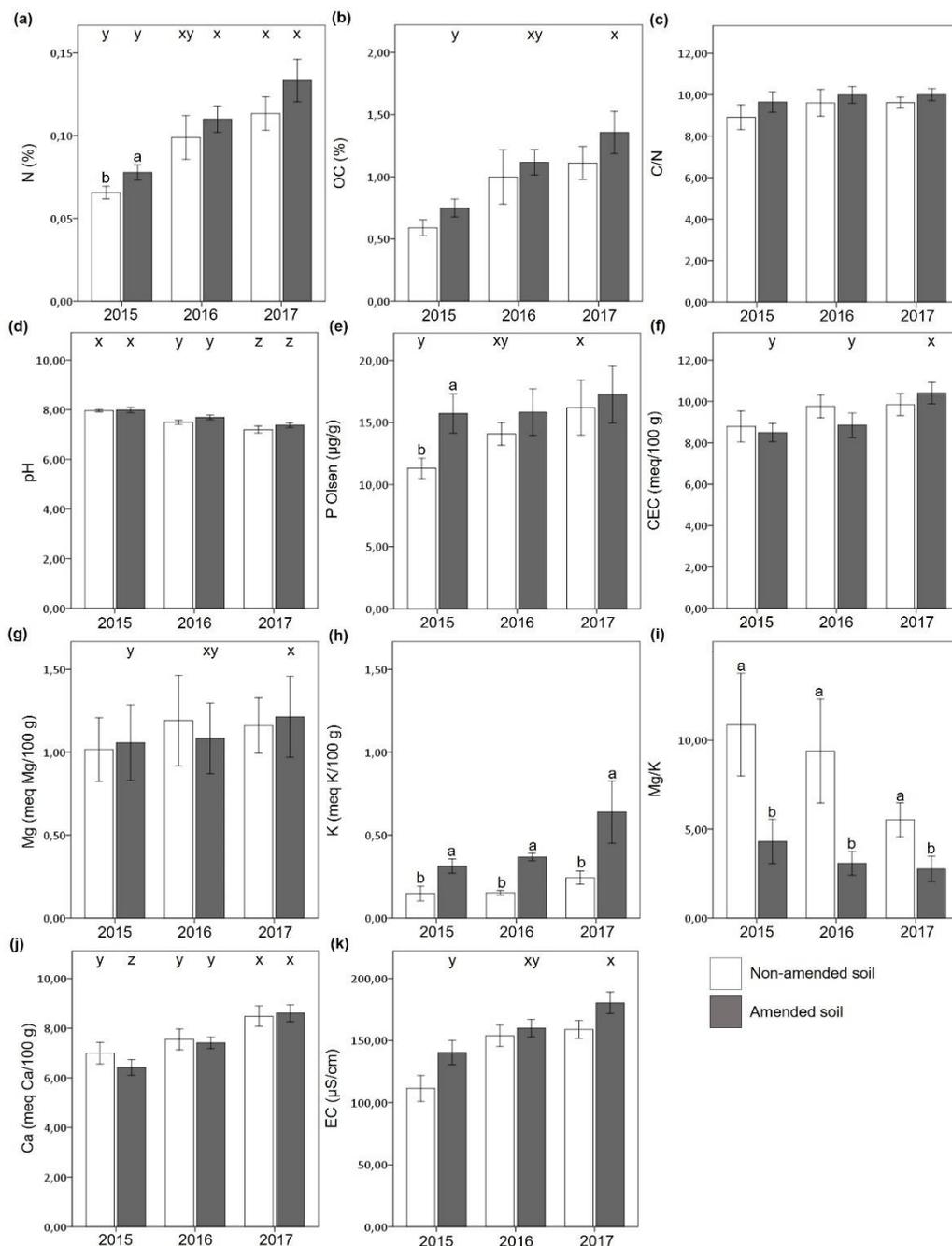
384 cover; (f) Annual spontaneous species cover; (g) Perennial spontaneous
385 species cover; (h) Annual ruderal species cover; (i) Perennial ruderal species
386 cover; (j) Typical grassland species cover; (k); Alien species cover. "a" and "b"
387 indicate significant differences between soil treatments ($p < 0.05$). "x", "y", and "z"
388 indicate significant differences among years ($p < 0.05$). Letters are not shown
389 when differences are not significant ($p \geq 0.05$). Data are presented as mean \pm
390 standard error.

391

392 **3.4 Soil chemistry**

393 Zeolite influenced N content in 2015 (Fig. 7a), whereas exchangeable K (Fig.
394 7h), and Mg/K (Fig. 7i) in the three study years, with higher values of N and
395 exchangeable K in the amended treatment and higher values of Mg/K in the
396 non-amended treatment. The amendment had no effect on the other considered
397 soil chemical parameters (Fig. 7).

398 From 2015 to 2017 N (Fig. 7a), CEC (Fig. 7f), exchangeable Ca (Fig.7) and EC
399 (Fig. 7k) significantly increased in both non-amended and amended soil, while
400 OC (Fig. 7b) and P Olsen (Fig. 7e) increased only in the amended one. pH
401 decreased from 2015 to 2017, independently of soil treatments (Fig. 7d), while
402 C/N, exchangeable K, Mg/K did not change over time (Fig. 7 c, h, and i).



403

404 **Fig. 7.** Soil properties over time in non-amended and amended soil. (a) N
 405 content; (b) organic C - OC content; (c) C/N ratio; (d) pH; (e) P Olsen; (f) cation
 406 exchange capacity - CEC; (g) exchangeable Mg content; (h) exchangeable K
 407 content; (i) Mg/K ratio; (j) exchangeable Ca content; (k) electrical conductivity -
 408 EC

409 “a” and “b” indicate significant differences between soil treatments ($p < 0.05$).
410 “x”, “y”, and “z” indicate significant differences among years ($p < 0.05$). Data are
411 presented as mean \pm standard error.

412

413 **4. Discussion**

414 The short-term results of our restoration experiment showed that both abiotic
415 (i.e. zeolite addition, climatic conditions) and biotic agents (i.e. species-specific
416 functional traits, plant-plant interaction) affected seedling performances,
417 herbaceous vegetation and soil properties dynamics.

418 Among the many properties of zeolites the prominent ones include an increase
419 in soil CEC, acting as a reservoir of K^+ (Hershey et al., 1980). Although natural
420 zeolite-enriched soils usually increase CEC by 30-40% (Jakkula et al., 2018) we
421 did not observe a significant increase probably because the amendment may
422 have a more evident and beneficial role in poorer soils (Wiedenfeld, 2003).
423 However, in agreement with other authors, this soil amendment increased N, P
424 Olsen, and exchangeable K (Ramesh and Reddy, 2011; Sangeetha and
425 Baskar, 2016). Zeolite negatively affected herbaceous layer height and ruderal
426 perennial species cover and positively influenced alien species cover and
427 hawthorn growth. The amendment increased soil moisture (only in 2016 and
428 2017), confirming that in certain conditions it can improve water holding
429 capacity (Ippolito et al., 2011; Ghazavi, 2015). However, in contrast with our
430 expectations, it had no impact on any other parameters. Unexpectedly, zeolite
431 effects declined before the end of our experiment. Other factors (such as
432 extreme weather events) were probably more important for the results of this

433 restoration project and may have had a stronger influence on vegetation
434 dynamics and soil properties.

435 The summer of 2015 was hotter and drier than average (as reported by Orth et
436 al., 2016), and affected topsoil microclimatic conditions. In comparison with
437 2016 and 2017, higher temperature and lower soil volumetric moisture content
438 were recorded in 2015. The harsh climatic conditions during the first summer of
439 the experiment negatively affected the survival rate and growth of seedlings
440 planted the year before, i.e. in 2014. Extreme weather events, such as
441 heatwaves and drought that characterized summer 2015 in Central Europe,
442 which could become increasingly frequent due to climate change (IPCC, 2014;
443 Orth et al., 2016), severely affected vegetation dynamics and, particularly,
444 seedling survival in our experiment (Saccone et al., 2009). However, our results
445 showed that herbaceous species were slightly affected by the abiotic stress in
446 the first study year, as shown by their high total cover. The recorded differences
447 with the following two years, even if significant, were negligible from a biological
448 point of view, since total herbaceous species cover ranged from 72% to 100%.
449 These findings appear to be consistent with previous studies, indicating a good
450 resistance and resilience of the herbaceous layer to heatwaves, particularly
451 when drought is not too severe (Hoover et al., 2014; De Boeck et al., 2015).

452 The ability of the herbaceous layer to survive and recover after the first growing
453 season, combined with more suitable meteorological and pedoclimatic
454 conditions of summer 2016, resulted in positive changes in the herbaceous
455 composition: two years after the restoration, perennial spontaneous species
456 increased their cover to the detriment of annual, alien, and sown ones. This

457 increase could potentially ameliorate soil fertility, improving SOM content due to
458 their deep root systems, whose turnover results in an increase of OC and N in
459 the topsoil (Baer et al., 2002; Hütsch et al., 2002; Carter and Gregorich, 2010).
460 Accordingly, in our study site, OC and N contents had higher values in 2016
461 compared to 2015 and continued to increase in 2017, highlighting that plant
462 residues contribute to soil formation and evolution (Yao et al., 2009; Pawlik and
463 Šamonil, 2018). Indeed, the rapid increment of soil C (+57.9% from 2015 to
464 2016 and +16.65% from 2016 to 2017) assessed in our restoration experiment
465 and also reported by Laganriere et al. (2010) after afforestation, was probably
466 linked to herbaceous species root turnover (Rasse et al., 2005). Due to root
467 turnover, SOM loss, usually detected during the first five years after soil
468 preparation in afforestation projects (Korkanç, 2014), was contrasted, and soil C
469 recovery accelerated (e.g. Paul et al. (2002) observed that C content is
470 recovered about 30 years after afforestation).

471 Summer 2017 was the wettest of the study period but the air temperature was
472 higher than the average of the period and did not differ from 2015.
473 Consequently, soil microclimatic conditions showed temperatures comparable
474 with 2016, but low soil volumetric moisture, like in the 2015 summer drought.
475 Thus, even if rainfall was high, high air temperatures favoured
476 evapotranspiration, causing topsoil desiccation. Anyway, soil drought did not
477 alter herbaceous layer and soil nutrient dynamics.

478 Despite the dry topsoils, seedlings showed high vitality at the end of the third
479 study year. Other mechanisms, such as facilitation, may have contributed to the
480 creation of suitable conditions for vegetation development. It has been

481 demonstrated, for example, that redistribution of water through roots has a great
482 impact on soil hydrology. Using “hydraulic lift” plants can passively transport
483 water from deeper and wetter soil layers and release it into shallower and drier
484 ones, recharging upper layers during drought periods and enhancing nutrient
485 availability and uptake (Caldwell et al., 1998; Armas et al., 2012). Indeed, when
486 roots penetrate deep soil layers hydraulic lift is improved and soil desiccation
487 delayed, prolonging root life (Jackson et al., 2000; Espeleta et al., 2004; Liste
488 and White; 2008). Particularly, seedling survival may have been enhanced by
489 the presence of a well established herbaceous layer, with a high cover of deep-
490 rooted species, such as perennial ones. In addition to soil fertility improvement
491 and “hydraulic lift”, herbaceous species provide shade that contributes to
492 facilitating seedlings establishment. With their canopy cover, herbaceous
493 species decrease the incoming photosynthetically active radiation and reduce
494 soil temperature in comparison to bare ground, providing suitable microsites for
495 seedlings establishment (Cavieres et al., 2008; Koyama and Tsuyuzaki, 2013).

496 Herbaceous layer development may have helped tree species survival after the
497 first year, whereas the occurrence of heatwaves and drought resulted in severe
498 mortality. However, our study showed that seedlings response to biotic and
499 abiotic factors, in terms of growth, was species-specific. Particularly, hawthorn
500 appeared well adapted to the study site. In suitable microclimatic conditions (i.e.
501 in 2016 and 2017) it started growing immediately after plantation. Even if oak is
502 the dominant species in the oak-hornbeam forest native to the area (i.e. the
503 lowland oak-hornbeam Mesophytic deciduous forest), it showed the worst
504 performance in terms of growth. No evidence of elongation was observed in

505 either the first or second growing season, independently of microclimatic
506 conditions. Elm showed the most contrasting responses to weather variation,
507 with very slow growth during the first year after plantation, both under harsh and
508 more suitable climatic conditions. Elm probably needs more time than hawthorn
509 to recover from transplanting. All three species used in our restoration project
510 were native to the area. However, the recorded differences in their
511 performances suggest that their adaptability is influenced by specific functional
512 traits (Cadotte et al., 2015; Funk et al., 2008).

513 **5. Conclusion**

514 The results showed that factors other than zeolitite influenced the dynamics of
515 our restoration experiment. Proper mechanical site preparations may improve
516 grassland restoration success and its resilience to abiotic disturbances, such as
517 heatwaves and drought. Once established, the herbaceous layer could
518 ameliorate microsite conditions by enhancing soil organic matter, soil nutrients,
519 and microclimatic conditions. Particularly, herbaceous vegetation contributed to
520 increase soil OC to a high rate despite other authors reporting lower rates.
521 Thus, the presence of a well-established herbaceous layer helped seedling
522 growth and survival.

523 Zeolitite application positively influenced some soil properties (namely, N, P
524 Olsen and exchangeable K), accelerating soil recovery. Nevertheless, its
525 application weakly affected seedling dynamics, except for hawthorn, which grew
526 better in the amended soil when climatic conditions were suitable.

527 The well-established herbaceous layer fostered organic matter, whose
528 increment was as high as that reported by other authors after afforestation.

529 Following soil preparation, zeolite addition and herbaceous layer establishment
530 within the three years, soil properties increased and reached the levels required
531 by other Italian Regional guidelines for anthropogenic soil recovery (e.g. Curtaz
532 et al., 2013), indicating a positive and rapid trend. Together, these results
533 highlighted that some attributes recorded in our experiment, such as the
534 presence of indigenous herbaceous species, the resilience to disturbance, and
535 a suitable physical environment (SER, 2004) can indicate the achievement of
536 the restoration aim.

537 In order to improve the success of restoration projects changes in the species
538 composition, interactions between plants and soil properties and the influence
539 of biotic and abiotic factors on vegetation establishment have to be taken into
540 account

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