Effect of zeolitite addition on soil properties and plant establishment during forest restoration

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Effect of zeolitite addition on soil properties and plant establishment during forest restoration

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Abstract

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

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

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

Key words: site preparation; zeolitite; facilitation; grassland restoration; oak-hornbeam forest; seedling performances.

1. Introduction

The building of transport infrastructure, such as motorways and high-speed railways, entails damage, destruction, fragmentation and isolation of habitat (Andrews, 1990; Cuperus et al., 1999; Forman, 2000). Construction activities have been shown to severely affect vegetation and soil properties. Soil over-compaction by the movement of heavy machinery alters soil structure. Bulk density increases while soil permeability to air and water as well as water holding capacity are reduced (Czyż, 2004; Batey, 2009). In compacted soil, seedlings and saplings are more susceptible to drought, as the root system is prevented from quickly reaching deep and moist horizons (Bassett et al., 2005; Sinnett et al., 2008; Self et al., 2012; Cambi et al., 2018). Topsoil loss, its mixing with subsoil or construction waste during storage and replacement may reduce soil quality (DEFRA, 2009). Vegetation and litter removal also contribute to the deterioration of soil properties. A lack in the provision of organic matter influences soil aggregate stability, soil porosity, gas exchange reactions and water relations (Schoenholtz et al., 2000).

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

In the restoration of degraded systems, the use of slow release amendments that produce long-term effects on soil properties, such as zeolitites, should be effective (Ming and Allen, 2001; Tan et al., 2018). Zeolitites are rocks with a content of zeolites, hydrated alkaline aluminosilicates, greater than 50%. The infinite, open, three-dimensional structure of zeolites, gives them the capacity to retain a large amount of water (Xiubin and Zhanbin, 2001) and they also have a high and selective cation exchange capacity (CEC). Zeolitites uptake macro and micronutrients such as potassium (K), calcium (Ca), sodium (Na), magnesium (Mg) and nitrogen (N) (Polat et al., 2004; Ozbahce et al., 2015), retain beneficial nutrients in the root zone and improve nutrients availability for plants.

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

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

2. Materials and methods

2.1 Study site.

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

The area was a road constructer’s yard, during the building of the motorway. Contrary to other yards located along the Corridor, where topsoil was stripped away and stockpiled, here it was never removed. The area was used to stock soil from other sites and inert waste. In November 2014, after the roadworks ended, the area was restored. Using a tractor, the soil was ripped (to a 70 cm depth) and ploughed (to a 40 cm depth) to reduce compaction caused by the movement of heavy machinery and stockpiling. In the topsoil (10 cm depth) the texture was sandy-loam. The pH was moderately alkaline (pH measured in H_2O ranged from 7.4 to 8.0). Soil organic matter (SOM) content in the topsoil ranged from 1.27% to 2.58% while the N content ranged between 0.07% and 0.15%. The CEC ranged between 6.94 and 9.62 meq/100 g while P Olsen and K ranged from 8.25 to 12.43 µg/g and from 0.20 to 0.80 meq/100 g, respectively. No trees or herbaceous cover were present when the experiment started. Tree and shrub seedlings (two-year-old containerized nursery plants) were therefore planted twice, in November 2014 and November 2015, while a grassland species mixture was hand sown in spring 2015. The seedlings used in the experiment were typical species of the lowland oak-hornbeam Mesophytic deciduous forest which represents the natural late seral forest ecosystem of the Po Plain, dominated by *Q. robur* and *Carpinus betulus* L. The grassland species mixture was composed of 10% *Lolium perenne* L. (perennial ryegrass), 40% *Festuca* gr. *rubra* L. (red fescue), 15% *Poa pratensis* L. (Kentucky bluegrass), 25% *Lotus corniculatus* L. (bird’s-foot trefoil), and 10% *Trifolium repens* L. (white clover). Grassland species were chosen among those that can contrast the presence of invasive herbs due to their competitiveness and longevity (Barker, 1995).

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

2.2 Experimental design

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

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

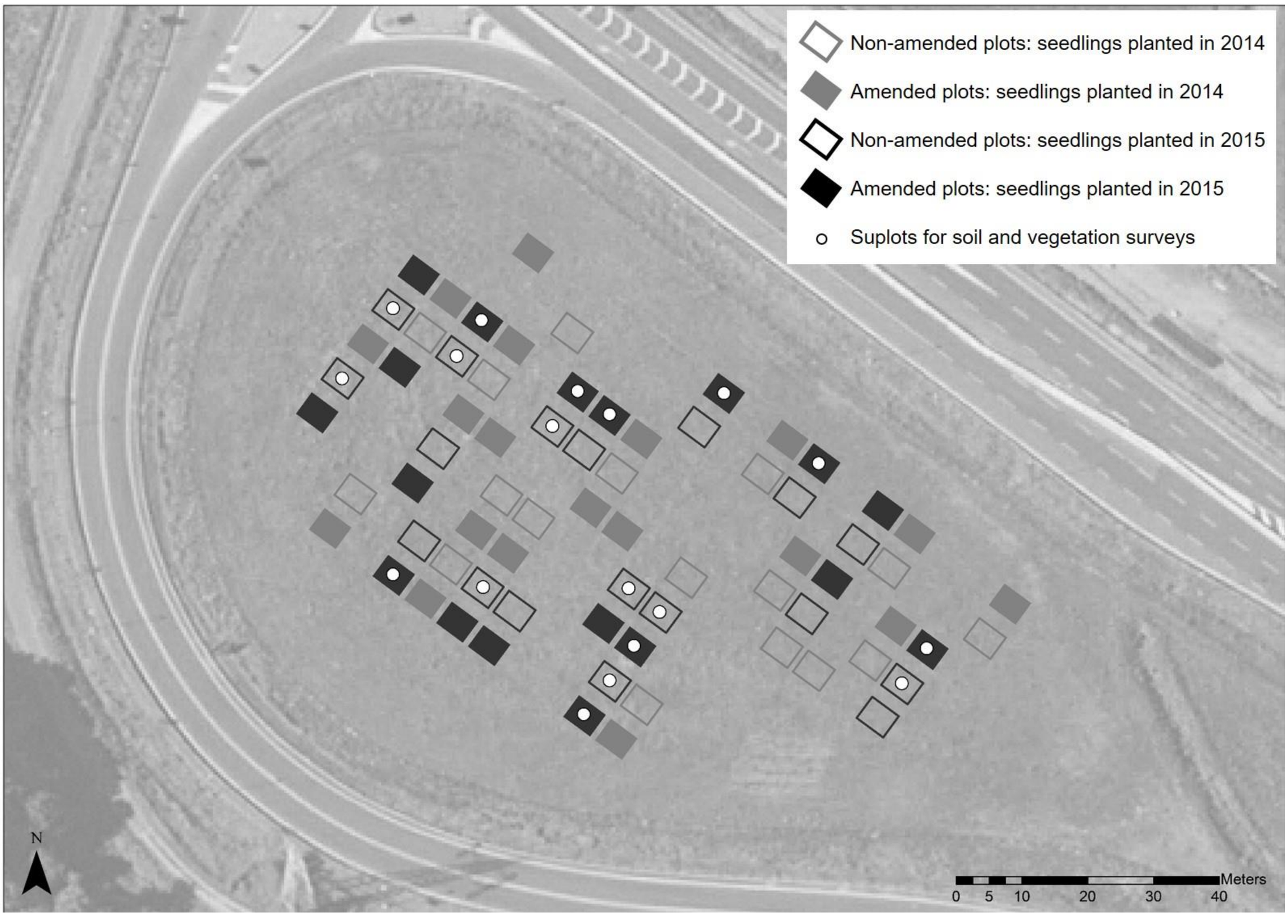

Fig. 1. Experimental design. Rows non-amended (no colour) alternated with those amended with zeolitite (fully coloured). The grey plots were planted in November 2014 and the black ones in November 2015. White circles indicate the plots in which we conducted soil and vegetation surveys. A motorway surrounded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

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

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

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

2.3 Data collection

2.3.1 Ancillary measurements

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

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

2.3.2 Seedlings

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

2.3.3 Herbaceous vegetation

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

2.3.4 Soil sampling and laboratory analysis

After the initial mechanical preparation and zeolitite addition, soil samples (topsoil, 0-10 cm depth) were collected in the middle of all the 18 sub-plots already used for vegetation surveys, once a year in late June. All samples were dried, sieved, and analyzed for the determination of pH, CEC, electrical conductivity (EC), exchangeable base content (Mg, K, Ca), total carbon (TC), inorganic carbon (IC), organic carbon (OC), and total nitrogen (TN). pH and EC were measured in water (soil:water = 1:2.5). The CEC was measured by the barium chloride extraction (pH 8.1) method.. Exchangeable base content and saturation, on the barium chloride extracts, were measured by AAS (flame atomic absorption spectrometer, Analyst 400, Perkin Elmer, Waltham, MS, USA). For the analysis of TC and TN, soil aliquots were milled and analyzed by dry combustion with a CN elemental analyzer (CE Instruments NA2100, Rodano, Italy). IC represented by the carbonate content was determined with a Dietrich Fruhling pressure-calcimeter. Total organic carbon (TOC) was calculated from the difference between TC and IC. Bicarbonate extractable phosphorus (P Olsen), assumed to represent readily bioavailable phosphate, was determined according to Olsen et al. (1954) with P detection by molybdate colorimetry (Murphy and Riley, 1962).

2.4 Data analysis

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

Assumptions of normality and homogeneity of variance were checked ($p \geq 0.05$) with Shapiro-Wilk and Levene tests, respectively. Variables that were not normally distributed were log-transformed prior to further statistical analysis. When normal distribution or homogeneity of variances were not met, even after log-transformation, the non-parametric Kruskal-Wallis test was used. When significant differences were found, Tukey's and Mann-Whitney's *post hoc* tests were performed for ANOVAs and Kruskal-Wallis tests, respectively.

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

Grassland species diversity was assessed for each sub-plot by calculating species richness (i.e. the total number of recorded species) and Shannon diversity index (Magurran, 1988). Each plant species was associated to its phytosociological optimum (at class level) according to Aeschimann et al. (2004). This approach has been widely used to evaluate vegetation response to management and disturbance regimes in different ecosystems (Lonati et al., 2013; Moris et al., 2017; Orlandi et al., 2016; Pittarello et al., 2016; Vacchiano et al., 2016). Therefore, according to their phytosociological optimum, plant species were grouped into three vegetation units, following an increasing gradient of habitat naturalness: i) annual ruderal species (i.e. species belonging to *Stellarietea mediae* class), ii) perennial ruderal species (i.e. species belonging to *Agropyretea intermedii-repentis* and *Artemisietea vulgaris* classes), and iii) typical grassland species (i.e. species belonging to *Molinio-Arrhenatheretea* class). The percentage cover of the following herbaceous species assemblages was then calculated for each plot: 1) sown species; 2) total spontaneous species (i.e. species not belonging to the sown mixture) divided into annual and perennial (according to Landolt et al., 2010); 3) annual and perennial ruderal spontaneous species; 4) typical grassland species; 5) alien species (i.e. the cumulative cover of *Ambrosia artemisifolia* L., *Artemisia verlotorum* Lamotte, *Erigeron annus* (L.) Pers., *Helianthus tuberosus* L., *Lepidium virginicum* L., *Oxalis fontana* Bunge, *Solidago gigantea* Aiton., *Thlaspi arvense* L., *Veronica persica* Poiret).

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

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

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

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

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

3. Results

3.1 Meteorological and pedoclimatic conditions

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

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

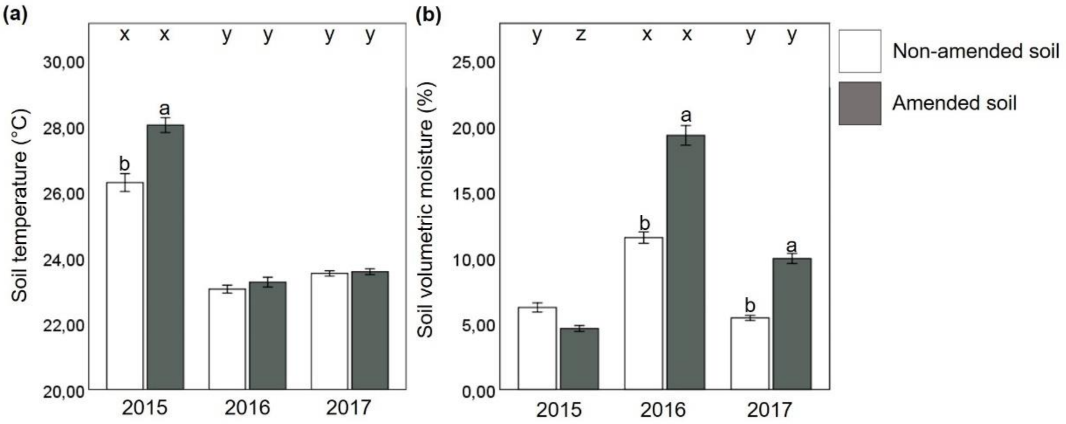

Fig. 2. Mean soil temperature (a) and volumetric moisture (b) during the three summer seasons (J, J, A). “a” and “b” indicate significant differences between treatments ($p<0.05$). “x”, “y”, and “z” indicate significant differences among years for each treatment ($p<0.05$). Letters are not shown when differences are not significant ($p\geq0.05$). Data are presented as mean ± standard error.

3.2 Seedlings

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

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

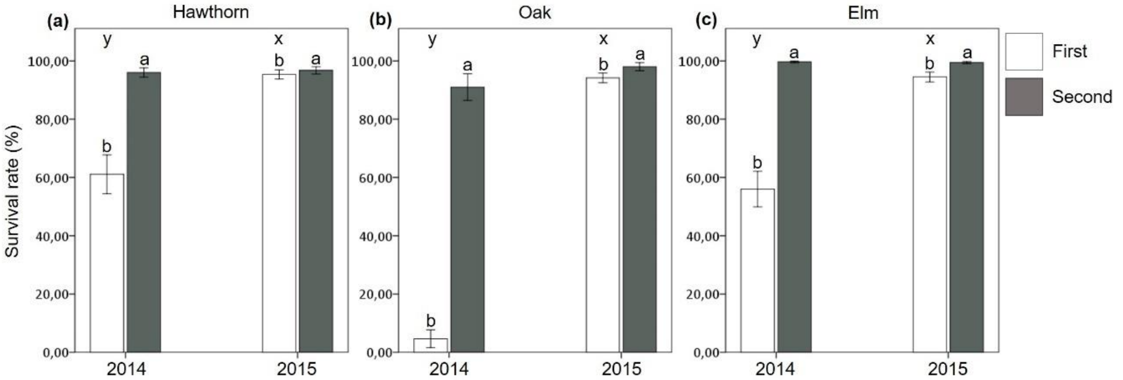

Fig. 3. Survival rate of (a) *C. monogyna* (hawthorn); (b) *Q. robur* (oak); (c) *U. minor* (elm) for each planting year (2014 and 2015) and growing season (First or Second). “a” and “b” indicate significant differences between first and second growing season (p<0.05). “x” and “y” indicate significant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

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

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

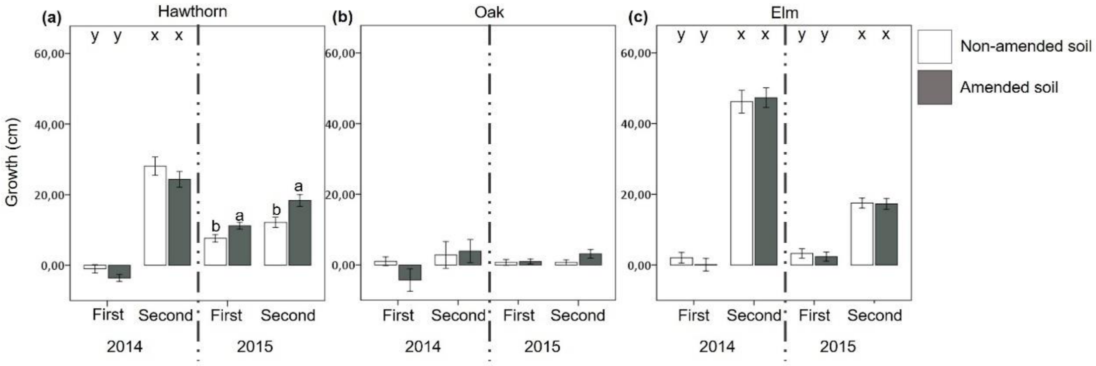

Fig. 4. Growth of (a) *C. monogyna* (hawthorn); (b) *Q. robur* (oak); (c) *U. minor* (elm) for the two planting years (2014 and 2015) and growing seasons (First and Second). “a” and “b” indicate significant differences between soil treatments ($p<0.05$). “x” and “y” indicate significant differences between the first and second growing season for each soil treatment ($p<0.05$). Letters are not shown when differences are not significant ($p\geq0.05$). Data are presented as mean ± standard error.

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

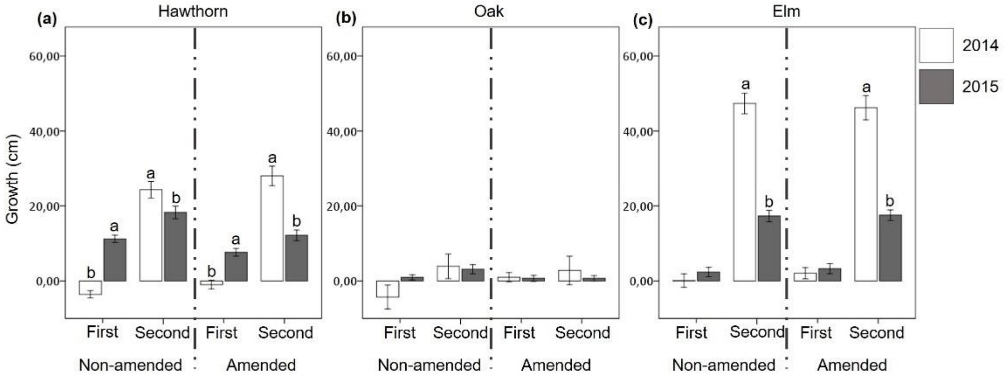

Fig. 5. Growth of (a) *C. monogyna* (hawthorn); (b) *Q. robur* (oak); (c) *U. minor* (elm) for the two soil treatments (non-amended and amended) and growing seasons (First or Second). "a" and "b" indicate significant differences between planting year for each growing season ($p<0.05$). Letters are not shown when differences are not significant ($p\geq0.05$). Data are presented as mean ± standard error.

3.3 Herbaceous vegetation

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

Year significantly affected all parameters, in both the non-amended and amended soil, except for annual spontaneous species cover in the amended soil (Fig. 6f). Species richness (Fig. 6b) and Shannon diversity index (Fig. 6c) decreased more from 2015 to 2017 in the non-amended than in the amended treatment. Herbaceous cover differed among years, with a progressive increase of perennial (spontaneous and ruderal, Fig. 6 g and i) species and typical grassland species (Fig. 6j) cover while sown species (Fig. 6e) and annual (spontaneous and ruderal, Fig. 6 f and h) species cover decreased.

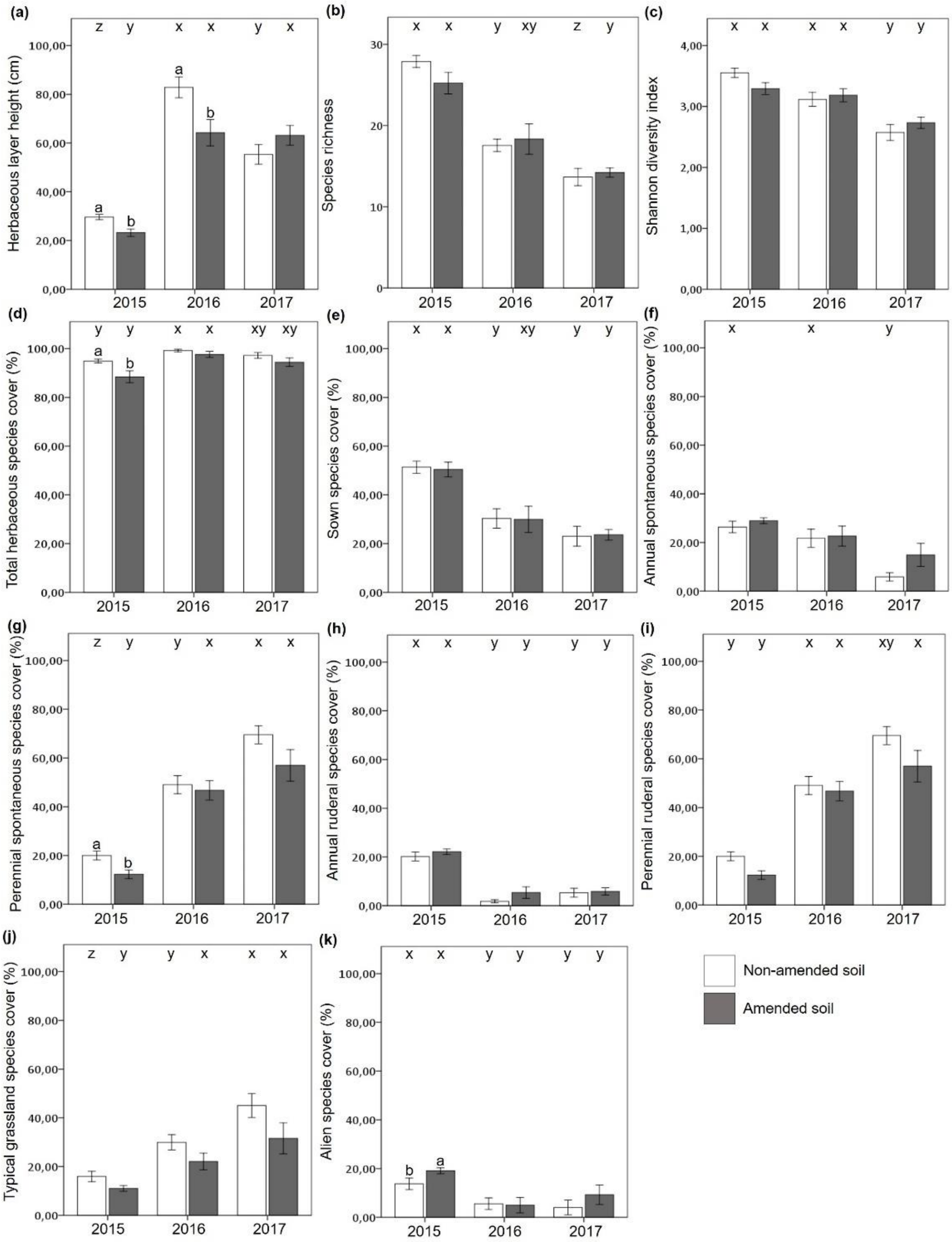

Fig. 6. Herbaceous cover dynamics in non-amended and amended treatments during the three study years. (a) Herbaceous layer height; (b) Species richness; (c) Shannon diversity index; (d) Herbaceous species cover; (e) Sown species

cover; (f) Annual spontaneous species cover; (g) Perennial spontaneous species cover; (h) Annual ruderal species cover; (i) Perennial ruderal species cover; (j) Typical grassland species cover; (k); Alien species cover. “a” and “b” indicate significant differences between soil treatments ($p<0.05$). “x”, ”y”, and “z” indicate significant differences among years ($p<0.05$). Letters are not shown when differences are not significant ($p≥0.05$). Data are presented as mean ± standard error.

3.4 Soil chemistry

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

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

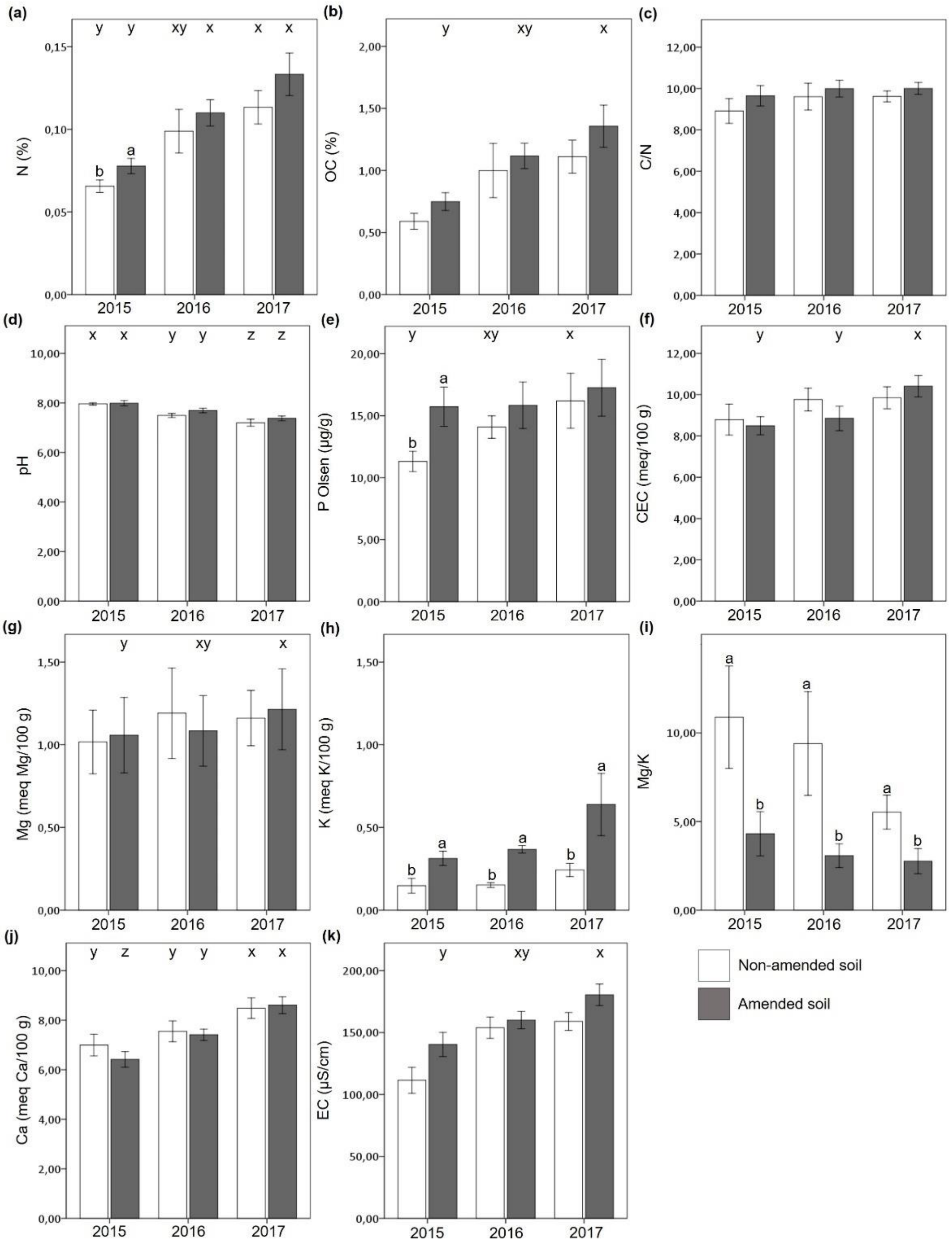

Fig. 7. Soil properties over time in non-amended and amended soil. (a) N content; (b) organic C - OC content; (c) C/N ratio; (d) pH; (e) P Olsen; (f) cation exchange capacity - CEC; (g) exchangeable Mg content; (h) exchangeable K content; (i) Mg/K ratio; (j) exchangeable Ca content; (k) electrical conductivity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

"a" and "b" indicate significant differences between soil treatments ($p<0.05$). "x", "y", and "z" indicate significant differences among years ($p<0.05$). Data are presented as mean ± standard error.

4. Discussion

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

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

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

The ability of the herbaceous layer to survive and recover after the first growing season, combined with more suitable meteorological and pedoclimatic conditions of summer 2016, resulted in positive changes in the herbaceous composition: two years after the restoration, perennial spontaneous species increased their cover to the detriment of annual, alien, and sown ones. This increase could potentially ameliorate soil fertility, improving SOM content due to their deep root systems, whose turnover results in an increase of OC and N in the topsoil (Baer et al., 2002; Hütsch et al., 2002; Carter and Gregorich, 2010). Accordingly, in our study site, OC and N contents had higher values in 2016 compared to 2015 and continued to increase in 2017, highlighting that plant residues contribute to soil formation and evolution (Yao et al., 2009; Pawlik and Šamonil, 2018). Indeed, the rapid increment of soil C (+57.9% from 2015 to 2016 and +16.65% from 2016 to 2017) assessed in our restoration experiment and also reported by Laganiere et al. (2010) after afforestation, was probably linked to herbaceous species root turnover (Rasse et al., 2005). Due to root turnover, SOM loss, usually detected during the first five years after soil preparation in afforestation projects (Korkanç, 2014), was contrasted, and soil C recovery accelerated (e.g. Paul et al. (2002) observed that C content is recovered about 30 years after afforestation).

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

Despite the dry topsoils, seedlings showed high vitality at the end of the third study year. Other mechanisms, such as facilitation, may have contributed to the creation of suitable conditions for vegetation development. It has been demonstrated, for example, that redistribution of water through roots has a great impact on soil hydrology. Using “hydraulic lift” plants can passively transport water from deeper and wetter soil layers and release it into shallower and drier ones, recharging upper layers during drought periods and enhancing nutrient availability and uptake (Caldwell et al., 1998; Armas et al., 2012). Indeed, when roots penetrate deep soil layers hydraulic lift is improved and soil desiccation delayed, prolonging root life (Jackson et al., 2000; Espeleta et al., 2004; Liste and White; 2008). Particularly, seedling survival may have been enhanced by the presence of a well established herbaceous layer, with a high cover of deep-rooted species, such as perennial ones. In addition to soil fertility improvement and “hydraulic lift”, herbaceous species provide shade that contributes to facilitating seedlings establishment. With their canopy cover, herbaceous species decrease the incoming photosynthetically active radiation and reduce soil temperature in comparison to bare ground, providing suitable microsites for seedlings establishment (Cavieres et al., 2008; Koyama and Tsuyuzaki, 2013).

Herbaceous layer development may have helped tree species survival after the first year, whereas the occurrence of heatwaves and drought resulted in severe mortality. However, our study showed that seedlings response to biotic and abiotic factors, in terms of growth, was species-specific. Particularly, hawthorn appeared well adapted to the study site. In suitable microclimatic conditions (i.e. in 2016 and 2017) it started growing immediately after plantation. Even if oak is the dominant species in the oak-hornbeam forest native to the area (i.e. the lowland oak-hornbeam Mesophytic deciduous forest), it showed the worst performance in terms of growth. No evidence of elongation was observed in either the first or second growing season, independently of microclimatic conditions. Elm showed the most contrasting responses to weather variation, with very slow growth during the first year after plantation, both under harsh and more suitable climatic conditions. Elm probably needs more time than hawthorn to recover from transplanting. All three species used in our restoration project were native to the area. However, the recorded differences in their performances suggest that their adaptability is influenced by specific functional traits (Cadotte et al., 2015; Funk et al., 2008).

5. Conclusion

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

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

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

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

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

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