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Primary vegetation succession and the serpentine syndrome: the proglacial area of the Verra Grande Glacier, North-Western Italian Alps

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Summary

Aims

Initial stages of pedogenesis are particularly slow on serpentinite. This implies a slow accumulation of available nutrients and leaching of phytotoxic elements. Thus, a particularly slow plant primary succession should be observed on serpentinitic proglacial areas. The observation of soil-vegetation relationships in such environments should give important information on the development of the "serpentine syndrome".

Methods

Plant-soil relationships have been statistically analysed, comparing morainic environments on pure serpentinite and serpentinite with small sialic inclusions in the North-western Italian Alps.

Results and Conclusions

Pure serpentinite supported strikingly different plant communities in comparison with the sites where the serpentinitic till was enriched by small quantities of sialic rocks. While on the former materials almost no change in plant species composition was observed in 190 years, 4 different species associations were developed with time on the other. Plant cover and biodiversity were much lower on pure serpentinite as well. Extremely low P and high Ni contents in soil were strongly related with these differences, but none of them could be interpreted as the actual limiting factor for plant development on pure serpentinite. Other nutrients or bases were not related with the different primary succession speed and species composition.

Keywords

Endemic species; Glacier forefield; Moraines, Nickel; Phosphorus

1. Introduction

Introduction

Glacier retreat is one of the most visible effects of climate change, which, in the European Alps, continued with only few interruptions since the end of the Little Ice Age (LIA), around 1820-1860 (Ivy-Ochs et al. 2009). Habitats characterized by different ages coexist over short distance in the deglaciated surfaces, substituting space for time, with little effect of other geo/climatic factors otherwise normally observed. Therefore, these surfaces offer the opportunity of observing the time dependence of soil and ecosystem properties and, if lithological differences exist, to stress the effect of soil parent material.

The soil parent material has a prominent importance for plant growth especially at the early stages of soil development and plant succession (Rajakaruna and Boyd 2008). Serpentinitic parent materials have a strong effect on soil fertility and thus significantly affect the structure of plant communities. "Serpentine soils" are typically characterized by unique chemical and physical properties that reduce plant productivity and induce stress and toxicity in non-adapted species (the so called "serpentine syndrome") (Jenny 1980). These unique stressful chemical and physical properties include a low Ca:Mg ratio, low nutrient concentrations (N, P, K), abundant heavy metals (Ni, Cr, Co), proneness to drought and erosion (Brooks 1987). Consequently, plant communities growing on serpentine soils often show different species composition and high degree of endemism (e.g., Brooks 1987, Kazakou et al. 2008) when compared with other substrata. In particular, plant colonization on raw serpentinitic materials should be particularly difficult and much slower than on other substrata, as early weathering releases possibly toxic elements (such as Ni and/or excessive Mg), which can deeply impact the ecosystem development, but only small quantities of nutrients (Ca, P and K). In these conditions, the release of elements accumulated in plant debris may become essential for plant nutrition (Bonifacio et al., 2013).

On recently deglaciated serpentinite surfaces, pedogenic development is extremely slow (D'Amico et al. 2015), much slower than on other substrates in similar climatic conditions (e.g., Ugolini 1966; Burt and Alexander 1996; Righi et al. 1999; Andreis et al. 2001; Egli et al. 2006; D'Amico et al. 2014a, Temme et al. 2016). Scarce acidification, organic matter accumulation and mineral weathering hamper plant colonization, that remains scant even after 190 years since material deposition (D'Amico et al. 2015). Small quantities of gneiss in the parent till enhance the rate of pedogenesis, as shown by increased organic matter inputs, higher acidification rate and nutrient biocycling (D'Amico et al. 2015). This faster pedogenesis and a higher nutrient level at the beginning of soil formation allow a much faster vegetation encroachment with positive feedbacks on soil development.

Based on these results, our hypothesis was that even small sialic inclusions may affect vegetation development in a serpentinitic glacier forefield, and thus we aimed at investigating the factors influencing the establishment of vegetation and primary succession in harsh serpentine habitats by answering the following questions:

a) Are there any differences in vegetation succession along three soil chronosequences characterised by slight lithological variations?

b) Are the differences related to soil conditions, and which are the most important edaphic constraints at the beginning of colonisation?

c) Is there any amelioration in soil conditions through element leaching and biocycling with the ongoing of plant colonisation?

d) Which are the soil properties that mainly influence the presence or absence of common and endemic species?

2. Materials and methods

2.1. Study area

The Verra Grande glacier forefield is located in the upper Ayas Valley (Aosta Valley, Pennine Alps, Italy, Fig. 1). A precise dating of the Little Ice Age (LIA) Verra Grande moraine system is missing, but the most updated datings are reviewed by D'Amico et al. (2015), who studied three soil chronosequences in the area: one on the western moraine, one on the eastern part and one on the flatter basal till. Several events of moraine depositions are visible in the area that can be ascribed to different phases of deglaciation after the LIA glacial maximum. A further moraine could have been deposited after the 14^{th} Century advance, as it happened in the nearby Gorner Gletscher on the northern slope of Monte Rosa massif (Holzhauser et al. 2005), but also in other cold periods during the Late Holocene (i.e., ~ 3.0-2.3 ky ago), as it has been observed in a few glaciers in the Swiss Alps (Schimmelpfennig et al. 2014). Outside the proglacial area, soils developed on late glacial materials (i.e., ~ 11500 years ago). The glacial till is composed of antigoritic serpentinite, associated with lenses of chlorite-schists, talc-schists and traces of Ca-bearing minerals derived from ophicalcite and rodingite inclusions, belonging to the Zermatt – Saas ophiolite. The eastern lateral moraines are enriched with small amounts (<10% in volume) of granitic-gneissic clasts, derived from Monte Rosa Nappe outcrops (Mattirolo et al. 1951).

Present-day natural timberline is around 2400 m a.s.l., and the sampled sites lie in the upper subalpine belt. The climate of the Ayas valley is inner-alpine, continental, with rather low yearly rainfall. In Champoluc (1450 m a.s.l., 5 km far from the study area), the mean precipitation is 730 mm/y, well distributed throughout the year; the average July rainfall is around 60 mm. Slightly higher values are expected in the proglacial area (Mercalli 2003). Drought stress is possible during some particularly dry summer seasons. The mean annual temperature is between 0 and +2°C (Mercalli 2003). The soils are normally weakly developed Skeletic Eutric Regosols (FAO-ISRIC, 2014), with initial podzolization locally on the ca. 700-3000 years old moraine (D'Amico et al. 2015).

2.2 Soil sampling and analysis, vegetation survey

We sampled 55 topsoils (0-10 cm) along the three different chronosequences identified by D'Amico et al., (2015): 19 sites on the western lateral moraines (W sites, pure serpentinite), 18 in the eastern ones (E sites, small gneiss inclusions), 18 in the flat basal till in the intramorainic area (C sites, pure serpentinite). Among these 55 sites, two are located in Late Glacial terrains (Younger Dryas) on the eastern and western lateral moraine systems, and represent the typical subalpine forest with *Rhododendron ferrugineum* L. understory (climax conditions). The altitude range of the sampled area is limited to the 2070-2320 m elevation belt: excessive steepness and the consequent erosion processes inhibit ecosystem and soil development above this altitude. The sampling sites are typical representative of most types of vegetation cover for the considered moraine systems.

At each site a phytosociological survey was carried out in homogeneous areas of 4 m^2, visually estimating the percent cover of each species; plant species were identified according to Pignatti (1992). Field description of site and soil profile characteristics was carried out according to FAO guidelines (2006).

The following data were collected (in brackets, measure unit and acronyms used from now on): altitude, slope steepness (slope, %), aspect (°), surface rockiness (SR, %), bare soil (NS, %), erosion and tree cover (tcov, calculated as percent area on a 100 m^2 surface). Aspect and slope steepness were combined into the heat load factor (HL), a proxy of potential solar radiation and potential evapotranspiration (McCune and Leon 2002). Erosion, surface rockiness, bare soil (NS) and tree cover (tcov) were determined by visual area estimation.

Approximately 0.5 kg of soil material (0-10 cm depth) was collected in each site. These samples were air-dried, sieved to 2 mm and analysed following the methods reported by Van Reeuwijk (2002). The pH was determined potentiometrically in water extracts (soil:solution=1:2.5). The total C and N concentrations were obtained by dry combustion with an elemental analyser (CE Instruments NA2100, Rodano, Italy). The carbonate content was measured by volumetric analysis of the carbon dioxide liberated by a 6 M HCl solution. Organic carbon (TOC) was then calculated as the difference between total C content measured by dry combustion and carbonate-C. Exchangeable K^+, Na^+, Ca^{2+}, Mg^{2+}, and Ni^{2+} (later on, K, Ca, Mg, Ni) were determined by flame atomic absorption spectroscopy (FAAS, Perkin Elmer 4000) after exchange with NH_4^+-acetate at pH 7.0. Available P (indicated as P) was determined by extraction with $NaHCO_3$ and detection by molybdate colorimetry. Considering the high solubility of both serpentine and P-bearing soil fractions, total P (Pt) and Ni (Nit) were extracted with aqua regia (1:3 solution of concentrated HNO_3:HCl) and detected respectively by molybdate colorimetry and FAAS.

2.3. Numerical analysis

All statistical elaborations were performed using R 3.0.1 software (R Foundation for Statistical Software, Institute for Statistics and Mathematics, Vienna, Austria).

In order to follow the succession of different plant communities on the 3 morainic systems (question a), vegetation types were classified using Cluster Analysis), average linkage, Bray-Curtis dissimilarity algorithm using the Vegan R package (Oksanen et al. 2011). Cluster stability was assessed through the "bootstrap" noise-adding and subsetting methods (Hennig 2007): if the resulting Clusterwise Jaccard mean is below 0.5, the cluster is considered "dissolved" and not significant, while it is regarded as "stable" and significant if the value is above 0.75. The number of clusters to be considered during the following analysis was chosen based on the ratio between the total number of clusters and the number of stable ones and according to their ecological significance. The bootstrap method was applied to a variable number of clusters (2-18).

In order to detect the edaphic constraints to plant colonization on pure serpentinite and the positive chemical effects of sialic inclusions (question b), differences in soil chemical properties between the three morainic systems were evaluated using a one-way analysis of variance (ANOVA). Tukey HSD was used to test differences at a significance level of $p < 0.05$, and the results were showing as boxplots using the multcomp R package (Hothorn et al. 2008). Canonical Correspondence Analysis (CCA, Ter Braak 1987) was used to highlight the important factors and constraints correlated with the vegetation gradients on the three moraine systems. We used biplot scaling focused on inter-species distances, without transforming species cover or downweighting rare ones. Sampling sites were labelled on the biplot with the number of the cluster they were included in, to show the dependence of plant communities on soil-environmental (causal) factors. The statistical significance was verified with Montecarlo permutation tests.

To check possible amelioration effects by plant communities and/or plant species (question c), we calculated the ratios between available and total elements, (P/Pt, Ni/Nit) and the Ca/Mg (able to show biocycling of Ca compared to Mg leaching), which can be considered proxies for biocycling or leaching of elements (e.g., Bonifacio et al., 2008). Then we compared these values in well vegetated and "barren" plant communities using ANOVA, and we added the ratio in the Random Forest elaborations for each species and plant community grouping (which didn't give significant results and whose results are not shown).

To detect the relationships between species (presence-absence data) and soil and site factors (question d), we used the Random Forest (RF) models (Breimann 2001), included in the RandomForest R library (Liaw and Wiener 2002) on all species growing in more than 5 study sites. We checked the optimal number of trees (ntree) reducing the out of bag error to a minimum, and then modified the number of trees to be extracted in the Random Forest from 500 to 1000. The optimal number of randomly selected variables (mtry) to be used in each step of the bootstrap process was also checked for each species. Positive or negative interactions between predictive variables obtained with Random Forest elaborations and each considered plant species (presence-absence data) were checked using Generalized Additive Models (GAM, Hastie and Tibshirani 1990, gam function, family "binomial"), using only the important variables for each considered species. GAM models do not assume any general shape of the response curve (Austin and Meyers 1996).

3. Results

3.1. Primary vegetation succession along the chronosequences

The vegetation cover and the species composition drastically differed on the three moraine systems (western and eastern lateral moraines and central basal till). The eastern lateral moraines had a complete plant cover with a high species richness (Nsp, fig. 2a), while the western ones were dominated by barren soil and stones (p-value < 0.01, not shown).

On the western moraines, even after 190 years since deposition, the main source of ground stabilization was provided by cryptogamic crusts.

No statistically significant chronofunction of species richness was obtained (fig. 3a). However, on the eastern system, species richness had an increasing trend until 1860, and then it decreased until reaching the climax subalpine forest. Species richness (Nsp) tended to increase with age on the western lateral moraines.

The cluster analysis evidenced a different species composition and speed of succession of plant communities along the primary successions in the three morainic systems. We selected a subdivision in 11 ecologically significant clusters, 5 of which were composed of just one sampling plot; the larger clusters were statistically stable, as shown by to the high Jaccard stability index (Electronic Annex, fig. 4).

The highest cluster division separated clusters 6 and 11 from the others. These two groups represented initial vegetation types, respectively on the eastern and western lateral moraines, colonized by different basophilous pioneer species (Landolt 1977) with only 2 species in common (Electronic Annex). Cluster 6 (E1) had a much higher species richness and total cover, did not support any serpentine-endemic and, despite the high erosive disturbance, included some intermediate grassland species together with pioneer ones (Caccianiga et al. 2006, Erschbamer et al. 2008). *Cardamine plumieri* and *Silene vulgaris* subsp. *glareosa*, showing the typical serpentine morphology (purple leaves and small dimensions), grew on raw pure serpentine till (cluster 11, site W1).

The second highest division separated clusters dominated by late successional or forest species from the others. In particular, cluster 10, 5 and 2 were located on the left hand side of this division. The first 2 represent, respectively, a mid-successional grassland site on the 1820 eastern lateral moraine and a wet site in the central area. Cluster 2 included most forested sites, together with pre-LIA climax subalpine forests, dominated by *Larix decidua*, *Pinus cembra*, *Rhododendron ferrugineum*, *Vaccinium* ssp., *Calluna vulgaris* and *Festuca varia*, usually enriched in some serpentine endemics such as *Thlaspi sylvium* and *Carex fimbriata*.

Early or mid-successional communities were located on the right hand side of the second highest subdivision. In this large group of clusters, plant communities located on the eastern lateral moraines (clusters 7, 8 and 9) were strongly differentiated from the central (cluster 3) and western ones (clusters 1 and 4). In particular, cluster 4 included almost all LIA sites on the western moraines except the initial pioneer community (cluster 11) and one 190-year-old barren site (W18), which belonged to cluster 1. The few larch stands on the western lateral moraines were associated with cluster 2. Cluster 4 was characterized by pioneer, early successional and serpentine endemic species. The presence of *Carex fimbriata* (serpentine endemic species on the Alps, Richard 1985) differentiated LIA surfaces deposited before 1920 from the 1920 and younger ones (where it was not found). Cluster 3 represented the most common vegetation type in the central, gently sloping area, characterized by a rather low plant cover dominated by *Dryas octopetala* accompanied by serpentine endemics and pioneer species.

Summarizing, plant communities on the eastern lateral moraines were attributed to 5 different clusters. The 1945 materials (E2) were colonized by a plant community grouped together with the western sites in cluster 4 despite the much higher plant cover. The moraines deposited between 1860 and 1920 had a higher species richness; a large number of species characteristic of intermediate succession stage and of stable basophilous grassland grew together with pioneer ones (clusters 4, 5, 8). The grasslands growing on the 1820 and older lateral moraines (cluster 9) had a lower species richness because of fewer pioneers and more species typical of acidic subalpine grassland; larch trees were more common as well as *Vaccinium* ssp. shrubs (cluster 2). The older moraine (likely 700/3000 years old) was covered by larch forest, but with a scattered ericaceous understory, different from the complete Rhododendron understory characterizing the Late Glacial surfaces (but both attributed to cluster 2).

Five vegetation types colonized flat intramorainic basal till. The youngest sites belonged to the cluster 4, together with the western sites. On 110 years old steeper slopes (C10) the vegetation was grouped with 190 years old western barren sites (cluster 1). Most other not forested sites were included in cluster 3, only found in this moraine system. Under dense larch tree stands older than 65 years, plant communities were grouped with the climax subalpine ones, despite the scarcity of *Rhododendron ferrugineum* (cluster 2). Cluster 7 (site C9) represented humid sites along streams.
A different speed of primary succession in the three moraine systems was thus observed (fig. 5).
Three strict serpentine endemics were found in the study area, growing in slightly different habitats (Electronic Annex): *Cardamine plumieri*, *Thlaspi sylvium* and *Carex fimbriata*. *Cardamine plumieri* usually grew in stony, barren, young or eroded soils (clusters 1, 4, 9, 11). *Thlaspi sylvium* (absent on initial soils) was most common in intermediate succession communities (high cover values in clusters 5, 7, 8, 9, 10); it was common also in barren sites (cluster 4) and climax forests, but its surface cover was here low. *Carex fimbriata* was most common in closed communities, associated with acidophilous species (cluster 2) or in barren sites older than 60-90 years. *Silene vulgaris* subsp. *glareosa* showed the typical serpentine morphology, with purple leaves and particularly small dimensions (Kazakou et al., 2008), as already noticed in nearby serpentine habitats (D'Amico and Previtali 2012; D'Amico et al. 2014b).

3.2. Soil properties, edaphic constraints and possible amelioration along the chronosequences

Many chemical parameters changed across the 3 different morainic systems (fig. 2). The western moraines (W), had the highest Nit and the lowest Pt contents of the whole proglacial areas, although Nit was not significantly different from the central sites (C). Available P was low as well in W sites. Other significantly different edaphic properties were the high exchangeable Ni contents (with intermediate values in the central sites) and rather low Ca levels. Interestingly, Mg was low in the western sites, but the difference was not significant. The Ca/Mg ratio showed a much wider range on the barren western lateral moraines than in the other sectors. The ratio between Ni and Nit (Ni/Nit, showing leaching) and P/Pt (indicating biocycling of P) did not significantly change in the considered forefield (fig. 2i and not shown, respectively).
A general age trend for stable Pt contents in the forefield was observed, followed by a slow decrease in sites older than 160 years old in the eastern and central sectors (fig. 3b). The more leachable Nit showed a significantly decreasing trend with age in both eastern and western lateral moraines (fig. 3c). Biocycling/leaching indicators (P/Pt and Ni/Nit ratios) did not show any age trends as well (data not shown).
The constrained ordination evidenced that the different plant communities were well separated across the space defined by the two main components, and were thus well associated with most of the selected edaphic and environmental properties (fig. 6, table 1); the whole model explained 40.6% of the species-environmental variance. In particular, Axis 1 was positively correlated with tree cover (which was significantly correlated with the C/N ratio) and age, and negatively with Ca/Mg molar ratio, pH, Ni and NS. As high pH and Ca/Mg ratios characterize young substrates thanks to ophicalcite inclusions, axis one can be interpreted mostly as a gradient of ecosystem development degree. Axis two clearly separates eastern sites (positive values), well positively correlated with nutrients (Pt, N, P) and negatively with Nit; it can thus be interpreted as a substrate gradient from pure serpentinite (negative values) to serpentinite with sialic inclusions.
No significant differences in the Ca/Mg and P/Pt ratios or Ni/Nit were measured under well developed and well vegetated plant communities (fig. 7), or under highly productive plant species, such as larch trees or Ericaceae. This means that no significant P or Ca bioaccumulation has occurred. Ni/Ni, conversely, was higher under barren vegetation.

3.3. Effect of soil properties on plant species and communities

The results obtained through the CCA analysis were confirmed by random forest and the GAMs (table 2), applied to bare soil communities (clusters 1, 3, 4, 11) which were characterized by low Pt, high Nit, low Mg and P, high Ni, low Ca and intermediate levels of Ca/Mg molar ratios. High Pt, low Nit, high Mg, age, P and Ca, low Ni and pH values were associated with well vegetated (clusters 2, 5, 6, 7, 8, 9, 10). Clusters 3 and 4 (respectively, common vegetation types with high barren soil on the basal till and western lateral moraines) were differentiated from each other by the good correlation with higher Mg and C/N ratio shown by the former (not shown). Eastern and western lateral moraines were differentiated by their opposite association with Pt and Nit.

pH values were important in differentiating the habitat of early-successional species (such as *Epilobium fleischeri*, *Poa minor*, *Salix breviserrata*. and *Trifolium pallescens*, preferring high pH) from late successional ones (i.e., *Anthoxanthum alpinum*, *Carex sempervirens*, *Festuca varia*, *Hieracium murorum* s.l., *Juniperus communis* and *Leonthodon helveticus*); low pH values were the most important factor correlated with the high cover by Ericaceae. Interestingly, serpentine endemic species such as *Carex fimbriata* and *Thlaspi sylvium* were associated with low pH values as well. Despite the significant negative correlation of P and Pt with plant communities with low plant cover, only four plant species (characteristic of advanced succession stages) were associated with high available P values (*Campanula scheuchzeri*, *Hieracium murorum*, *Leucanthemum vulgare*, *Orthilia secunda*). *Cardamine plumieri* (serpentine endemic common in screes, rock crevices and weakly developed soils), *Salix helvetica* and *Sempervivum montanum* were associated with low available P. Total P was correlated with some species, both positively (n=7, mostly late successional grassland species) and negatively (n=2, *Luzula lutea* and *Carex fimbriata*). High exchangeable Ni was an important factor positively involved in the distribution of 13 locally common species, including all serpentine endemics (*Carex fimbriata*, *Cardamine plumieri* and *Thlaspi sylvium*); six late successional grassland species were negatively correlated with this element. Total Ni was also positively or negatively correlated with many species (respectively, n=5, corresponding to pioneer species common in the western lateral moraines, and n=11, mainly corresponding to late successional species); some mid-successional grassland species were well correlated with intermediate Nit values (n=4), i.e., they showed a humped-back relationship with this element. High Mg was positively associated with the presence of some late successional species (n=8), including *Larix decidua* and *Thlaspi sylvium*, while it was negatively related with some early successional, basophilous ones (n=6). Exchangeable Ca was often associated with the same species, while the Ca/Mg molar ratio was correlated with only few species (3 positively, 2 negatively).

4. Discussion

4.1. Inhibited vegetation succession on serpentinite

In stable locations (including our post-glacial climax sites), the “serpentine syndrome” is often weakly visible on the Alps (D’Amico and Previtali 2012; D'Amico et al. 2014b), with serpentine plant communities being sometimes only enriched in endemic species.

Our study in the Verra Grande proglacial area provides clear evidences of the slow vegetation succession and pedogenesis characterizing serpentine habitats, when compared with other lithologies. In the Verra Grande forefield, much more than 200 years were needed to reach a climax vegetation on both western and eastern lateral moraines. Only small and scattered patches of quasi-climax plant communities were developed on near-flat surfaces younger than 150 years. Only the vegetation developed on the estimated 700-3000 years old basal till was quite similar to late glacial climax ones, despite the still weak surface acidification. This was an obvious evidence of the harsh edaphic properties related with the serpentine syndrome (Jenny 1980). The different speed of ecosystem evolution is particularly striking when compared with nearby alpine forefields (e.g. Lys glacier proglacial area, D'Amico et al. 2014a), where 90 years were enough for the establishment of a quasi-climax larch forest with a well-developed ericaceous understory layer, or with many studied proglacial areas in the world (e.g., Burga et al. 2010; Chapin et al. 1994; Ugolini et al. 1966).

By observing the differences between the three environments included in the Verra Grande forefield, it clearly emerges how small sialic inclusions in the serpentinite till increased the vegetation succession speed on young soils (question a). In fact, the plant colonization and the species turnover rate was much faster on the eastern moraines than in the opposite moraine system. On the pure serpentinitic till on the western lateral moraines, 190 years were not sufficient for the development of a complete vegetation cover. Here, most the observed vegetation plots (Fig. 4) were grouped in only one cluster (cluster 4), evidencing the inhibited species and communities substitution during the primary succession on pure serpentinite. Erosive processes, cryoturbation and proneness to drought were obviously other factors involved in the slow ecosystem development here, as normally happens in fresh morainic environments (e.g. Matthews and Whittaker 1987). However, the differences between the two lateral morainic systems cannot be explained by these factors, as surface disturbances associated with slope steepness were similar on both morainic systems in the earliest stages of ecosystem development. Slope related disturbances were quickly reduced only in the "more fertile" eastern side, characterized by a complete vegetation cover soon after deposition.

A very slow vegetation succession and pedogenic development characterized also soils on the basal till, on pure serpentinitic materials as well, except in the small patches colonized by larch trees and Ericaceae. In fact, three main different clusters were obtained on the central sites: a pioneer cluster associated to the vegetation developed on the western lateral moraines (cluster 4) observed in the youngest sites, one characterized by low plant cover and a low species richness, dominated by *Dryas octopetala* and serpentine endemic or indicator species, which dominated most of the LIA surfaces (cluster 3) and another, which included larch forest patches with some Ericaceae (cluster 2, less common, found on scattered surfaces older than ca. 90 years) and represented "safe sites" where small scale environmental properties, such as absence of erosion, favoured larch encroachment (Burga et al. 2011).

On the eastern moraines, where the serpentinitic till was enriched by small quantities of sialic clasts, a much faster change in species composition and cover through time was recorded. In particular, after 90 years the vegetation cover was complete. Plant communities were attributed here to five different clusters that showed the typical decrease of pioneer species and an increase in mid and late-successional ones. This primary succession had many species in common with the basophilous alpine one described by Raffl et al. (2006), with the addition of serpentine endemics. On the Verra Grande forefield, the time scale was rather extended and many early successionals persisted for much longer periods. Despite the higher speed of ecosystem development on the E sites, the change from grassland to subalpine forest needs more than 200 years on these surfaces, as only few ericaceous shrubs were beginning the colonization of 1821 moraines, but they were still rare in the larch forest growing on the 700-3000 BP one.

The species richness had different trends in the different sectors as well. On the eastern moraines, it approached the typical humped-back trend often observed in proglacial primary successions (e.g., Caccianiga et al. 2006; Carlson et al. 2010; Raffl et al. 2006), caused by the early colonization by pioneer species, a later co-presence of pioneer and late successional ones and a final disappearance of the first ones, associated with an increased competition for space and resources and the invasion by highly dominant late successional species (Matthews 1992). In the pure serpentine areas, species richness increased with substrate age, verifying the slower turnover in plant community composition and the weaker competition. Overall, species richness tended to increase from the western lateral moraine, the central basal till to the eastern sectors, verifying the lower plant diversity characterizing stressful habitats.

4.2. Development of the serpentine syndrome along the chronosequences: causes for serpentine infertility and species distribution

According to the soil analysis and the numerical elaborations, the different plant cover, plant species composition and succession speed between the different morainic environments cannot be associated with just one edaphic property (question b). However, many soil chemical characteristics can be excluded from list of the causal factors.

In particular, the scarcity of available Ca and a low Ca/Mg molar ratio have often been considered of primary importance in explaining the poor plant growth or the driving force for local adaptation of plants on serpentine soils (e.g., Brady et al. 2005; Kruckeberg 1954). Excessive Mg availability has been considered an important limiting factor as well (Nagy and Proctor 1997a). In the studied moraine habitats, the Ca/Mg ratio did not change according to sialic rocks content, nor amidst the different plant communities, thus it can be excluded from the driving factors of serpentine vegetation development. Both Ca and Mg were the lowest in the poorly developed barren soils located in the western lateral moraines, likely because of the lower CEC characterizing organic matter-poor soils. It is interesting to notice that, despite the biocycling and bioaccumulation normally characterizing Ca in surface horizons under closed vegetation on serpentinite (Bonifacio et al. 2013), the Ca/Mg molar ratio was particularly low below the most advanced successional stage (cluster 2), and it was higher in barren soils than in well vegetated ones. Excessive exchangeable Mg can thus be excluded as well from the list of limiting factors.

The CCA and random forest results show that pure serpentine soils developed on the western lateral moraines and on the basal till, characterized by low plant cover, were characterized by both high Ni (both Nit and Ni) and low P (both Pt and P). Nit and Pt had particularly strong and significant associations with plant communities growing on the different morainic sectors, and thus they likely had a stronger impact on vegetation than available elements on such weakly developed soils. Barren soils have often been reported to have smaller amounts of bioavailable Ni if compared with vegetated sites (Lazarus et al. 2011). However, in the Verra Grande forefield, the highest Ni contents were measured below pioneer communities and in barren soils, likely associated with the incipient weathering of Ni-rich serpentine minerals (Carter et al. 1987).

The extremely low total P contents in pure serpentinitic soils deposited during and after the LIA (commonly between 20-60 ppm in the western lateral moraine system, 20-120 in the flat basal till) likely caused particularly difficult conditions for initial plant encroachment and colonization, as shown in many boreal serpentine habitats (e.g., Carter et al. 1987; Nagy and Proctor 1997b). An enhancement in plant cover and productivity after P addition has often been observed in serpentine soils worldwide (Chiarucci 2004), and much evidence comes from European serpentine soils about a strong P-limitation for plant encroachment (Kazakou et al. 2010). On the eastern sector of the Verra Grande forefield, the contribution of a sufficient P content probably derived from the early weathering of sialic components, which contain some P-bearing apatite, may permit a fast, complete colonization by herbaceous species (Porder and Ramachandran 2013).

Ni had a specular trend compared with P; thus, their relative importance is difficult to discern. Many recent works state that Ni toxicity exists in many serpentine soils, but its importance is still a matter of debate (e.g., Brady et al. 2005; Chiarucci 2004; Kruckeberg 1992; Proctor 1997). For example, soil Ni did not have a significant effect on vegetation in many UK locations, despite extremely high concentrations (Carter et al. 1987; Proctor 1992). Likewise Ni did not appear to have a detrimental effect on vegetation in some subarctic environments (Dearden 1979). On the contrary, other works found that high Ni is sometimes able to critically reduce plant growth on serpentine soils and to reduce diversity of plant communities (Lee 1992; Robinson et al. 1996; Adamidis et al. 2013). In nearby ophiolitic environments, statistical elaborations showed that many common alpine species are excluded from serpentine soils mainly because of high exchangeable Ni (D'Amico and Previtali 2012). Thus, it cannot be excluded from the limiting factors for vegetation development on the pure serpentinitic till of the western and central morainic sectors.

From this study, we cannot obtain information on the inherent causes of serpentine endemisms or adaptation on the Alps, as there is not a comparison with nearby non-serpentine sites. However, the species distribution on serpentine, associated with particular edaphic gradients, can give important information on the edaphic requirements of the same species (question d). In particular, the importance of high exchangeable and/or total Ni in the distribution of serpentine endemics, such as *Cardamine plumieri*, *Carex fimbriata* and *Thlaspi sylvium*, and of other species preferentially found on Ni-rich serpentine soils such as *Luzula lutea* and *Salix breviserrata*, confirms the results obtained in other studies in the North-western Italian Alps (D'Amico and Previtali 2012). Some of these species were also associated with low P or Pt soil contents (i.e., *Carex fimbriata*, *Luzula lutea*, *Cardamine plumieri*). Other species appeared well correlated with high exchangeable Ni, but not with other characteristic properties of serpentine soils. In particular, *Minuartia verna* appeared positively influenced by high Ni, but also by low exchangeable Mg and intermediate P contents. This species is well adapted to colonize metal-rich soils in many parts of Central and Northern Europe (Baumbach 2012). *Minuartia laricifolia* was common in tree islands on young soils, and it was positively correlated with high Ni contents; this species appeared well correlated with Ni also in xeric, low altitude environments in the same Valle d'Aosta region (D'Amico et al. 2014b), and its adaptation to serpentine soils is well known (Moore and Kadereit 2013). Also in Mediterranean mountain ranges, many species appeared positively correlated with high Ni content (Tsiripidis et al. 2010), when different succession stages were considered. *Campanula cochleariifolia* was positively associated with high Ni and high Ca/Mg; this probably depends on the pioneer nature of this plant, which normally prefers rocky habitats on base-rich substrates. Other pioneer species, common on most substrata in the Alps, also seem well associated mostly with high exchangeable Ni (*Sempervivum* ssp., *Saxifraga* ssp. and *Trisetum distichophyllum*), but this could be related to the high Ni status of the most primitive soils on the western lateral moraines, which thus favoured pioneer species notwithstanding other edaphic factors. A few late successional species were negatively associated with Ni (either exchangeable or total). The same late-successional species were often well correlated with high total or available P.
The species-specific relationships with edaphic factors verify that different species are influenced, positively or negatively, by different chemical soil properties and are thus adapted to different components of the edaphic serpentine factor (Lazarus et al. 2011; Nagy and Proctor 1997b).

4.3. Fertility amelioration and facilitation along the serpentine soil chronosequence
A general trend towards amelioration of soil fertility was not verified at the 200 years time scale (question c). In particular, the Ca/Mg molar ratio tended to decrease along the primary succession. This means that only a weak Ca accumulation caused by biocycling has occurred in the Verra Grande Glacier forefield, and suggests that the encroachment of vegetation does not ameliorate soil chemical properties probably because of a little biological productivity in the young soil. Ca leaching associated with the weak acidification and to the ophicalcite-associated $CaCO_3$ dissolution was probably faster than Ca biocycling. The low Ca availability in pre-LIA soils was associated with the strong acidification and leaching in well-developed Podzols and Dystric Cambisols (as often observed in well-

developed acidic soils in the same region, D'Amico et al. 2008). A similar decline in the Ca/Mg ratio has been observed under 4 primary succession stages on raw serpentine soils in New Zealand (Lee and Hewitt 1982), but it was attributed to the release of large quantities of Mg from the dissolving parent minerals, under the attack or organic acids. In the same serpentine soils, Pt showed and increasing trend with age (Lee and Hewitt 1982). In the Verra Grande forefield, no bioaccumulation of P has been measured below the most productive plant communities, evidenced by the lack of differences in the P/Pt ratio under larch trees and Ericaceae compared to other vegetation types. Pt followed a decreasing trend in sites older than 190 years in the Pt-riches sites, while on pure serpentinite the values were stable, evidencing a counteraction to leaching by biocycling, while plant-fungi or plant-microbial communities biotic interactions (Carlson et al. 2010, Massaccesi et al. 2015) cannot be excluded as important factors involved in the progression of primary succession. Thus, the onset of climax vegetation in this serpentine environment does not seem to be related with a strong nutrient biocycling in the upper mineral soil horizons.

5. Conclusions

Different speed of soil development (D'Amico et al. 2015) characterize soil chronosequences on pure serpentine or where serpentine parent material is enriched in small amounts of sialic rocks; the same different speed of ecosystem development is shown by the different plant species turnover rate and pathways observed in the primary succession colonizing the two parent materials, despite the proximity. Ni and P appear as the main edaphic factors driving early stages of primary succession on serpentinite soils, and the two inherent causes of the serpentine syndrome on vegetation. These two properties, strongly dependant on parent material mineralogy and chemistry, drive primary succession, but with decreasing intensity until the climax vegetation that resembles the one developed on other substrates (but enriched with some endemic species). Thus, different "starting conditions" affect the whole primary succession for long times. The climax vegetation colonizes the proglacial area in small patches on surfaces younger than 190 years, on "safe sites" where larch trees have established, and becomes dominant only on surfaces between 3000 and 11500 years old. This is possibly related with the slow overcome of the edaphic factors of the serpentine syndrome, probably associated with leaching and biocycling in the organic layer. In fact, no significant increases of nutrients have been observed in surface mineral horizons. Another cause for the decreasing ecological effect of the serpentine edaphic properties could be related with the establishment of strong biotic interactions with increasing maturity of the ecosystem (Carlson et al., 2010). As no single element can be considered the actual limiting factor for plant life, we can conclude that serpentinitic substrates as a whole can be considered hard, with local differentiations from places where Ni excess or P scarcity are the most important limiting factors.

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References

Adamidis G C, Kazakou E, Baker A J M, Reeves R D, Dimitrakopoulo P G 2013 The effect of harsh abiotic conditions on the diversity of serpentine plant communities on Lesbos, an eastern Mediterranean island. Plant Ecol. Div. 7(3), 433-444.

Andreis C, Caccianiga M, Cerabolini B 2001 Vegetation and environmental factors during primary succession on glacier forelands: some outlines from the Italian Alps. Plant Biosyst. 135(3), 295-310.

Austin M P, Meyers J A 1996 Current approaches to modelling the environmental niche of eucalypts: implication for management of forest biodiversity. For. Ecol. Manag. 85, 95-106.

Baumbach H 2012 Metallophytes and metallicolous vegetation: evolutionary aspects, taxonomic changes and conservational status in Central Europe. In Perspectives on nature conservation—patterns, pressures and prospects. Ed. J Tiefenbacher pp 93–118. Tech, Rijeka.

Bonifacio E, Caimi A, Falsone G, Trofimov S Y, Zanini E, Goldbold D L 2008 Soil properties under Norway spruce differ in spruce dominated and mixed broadleaf forests of the Southern Taiga. Plant Soil 308(1), 149-159.

Bonifacio E, Falsone G, Catoni M 2013 Influence of serpentine abundance on the vertical distribution of available elements in soils. Plant Soil 368, 493-506.

Brady K U, Kruckeberg A R, Bradshaw Jr H D 2005 Evolutionary ecology of plant adaptation to serpentine soils. Ann. Rev. Ecol. Evol. Syst. 36, 243-266.

Breimann L 2001 Random Forests. Machine Learning 45, 15-32.

Brooks R R 1987 Serpentine and its vegetation: a multidisciplinary approach. Dioscorides, Oregon

Burga C A, Krüsi B, Egli M, Wernli M, Elsener S, Ziefle M, Fischer T, Mavris C 2010 Plant succession and soil development on the foreland of the Morteratsch glacier (Pontresina, Switzerland): straight forward or chaotic? Flora 205, 561-576.

Burt R, Alexander E B 1996 Soil development on moraines of Mendenhall Glacier, southeast Alaska. 2. Chemical transformations and soil micromorphology. Geoderma 72, 19–36.

Caccianiga M, Luzzaro A, Pierce S, Ceriani R M, Cerabolini B 2006 The functional basis of a primary succession resolved by CSR classification. Oikos 112, 10-20.

Carlson M L, Flagstad L A, Gillet F, Mitchell E A D 2010 Community development along a proglacial chronosequence: are above-ground and below-ground community structure controller more by biotic than abiotic factors? J. Ecol. 98, 1084-1095.

Carter S P, Proctor J, Slingsby D R 1987 Soil and vegetation of the Keen of Hamar serpentine, Shetland. J. Ecol. 75, 21-42.

Chapin F S, Walker L R, Fastie L, Sharman L C 1994 Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecol. Monog. 64(2), 149-175.

Chiarucci A 2004 Vegetation ecology and conservation on Tuscan ultramafic soils. Bot. Rev. 69, 252-268.

D’Amico M E, Julitta F, Previtali F, Cantelli D 2008 Podzolization over ophiolitic materials in the western Alps (Natural Park of Mont Avic, Aosta Valley, Italy). Geoderma 146, 129–136.

D’Amico M E, Previtali F. 2012 Edaphic influences on ophiolitic substrates on vegetation in the Western Italian Alps. Plant Soil 351, 73-95.

D'Amico ME, Freppaz M, Filippa G, Zanini E (2014a) Vegetation influence on soil formation rate in a proglacial chronosequence (Lys Glacier, NW Italian Alps). Catena 113:122-137.

D'Amico M E, Bonifacio E, Zanini E 2014b Relationships between serpentine soils and vegetation in a xeric inner-Alpine environment. Plant Soil 376, 111-128.

D'Amico M E, Freppaz M., Leonelli G., Bonifacio E, Zanini E 2015 Early stages of soil development on serpentinite: the proglacial area of the Verra Grande Glacier, Western Italian Alps. J. Soils Sediments 15, 1292-1310.

Dearden P 1979 Some factors influencing the composition and location of plant communities on a serpentine bedrock in Western Newfoundland. J. Biogeogr. 6(1), 93–104.

Egli M, Wernli M, Kneisel C, Haeberli W 2006 Melting glaciers and soil development in the proglacial area Morteratsch (Swiss Alps): I. Soil type chronosequences. Arct. Antarct. Alp. Res. 38(4), 499-509.

Erschbamer B, Sclag R N, Winkler E 2008 Colonization processes on a central alpine glacier foreland. J. Veg. Sci. 9(6), 855-862.

FAO 2006 Guidelines for Soil Description. 4th ed. FAO, Rome.

FAO-ISRIC 2014. World reference base for soil resources 2014. World Soil Resources Reports No. 103. FAO, Rome.

Hastie T J, Tibshirani R J 1990 Generalized additive models. Monographs of Statistics and Aplied Probability 43.

Holzhauser H, Magny M, Zumbuhl H J 2005 Glacier and lake-level variations in west-central Europe over the last 3500 years. The Holocene 15(6). 789-801

Hothorn T, Bretz F,Westfall P (2008) Simultaneous Inference in General Parametric Models. Biometr J 50(3), 346–363

Ivy-Ochs S, Kerschner H, Maisch M, Christl M, Kubik P W, Schluechter C 2009 Latest Pleistocene and Holocene glacier variations in the European Alps. Quat. Sci. Rev. 28, 2137–2149.

Jenny H 1980 The Soil Resource: Origin and Behavior. Ecol. Stud. 37, 256–59.

Kazakou E, Dimitrakopoulos PG, Baker AJM, Reeves RD, Troumbis AY (2008) Hypothesis, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. Biol. Rev. 83, 495-508.

Kazakou E, Adamidis G C, Baker A J M, Reeves R D, Godino M, Dimitrakopoulos P G 2010 Species adaptation in serpentine soils in Lesbos Island (Greece): metal hyperaccumulation and tolerance. Plant Soil 332(1), 369-385.

Kruckeberg A R 1954 The ecology of serpentine soils. III. Plant species in relation to serpentine soils. Ecology 35, 267-274.

Kruckeberg A R 1992 Plant life of western North American ultramafics. In: Robers BA, Proctor R (Eds.): The ecology of areas with serpentinized rocks. A world view. Kluwer, Dordrecht, pp 31-73.

Landolt E 1977 Ökologische Zeigerwerte zur Schweizer Flora, 64. Veröffentlichungen des Geobotanischen Institutes der Eidgenössischen Technischen Hochschule, Stiftung Rübel, Zürich.

Lazarus B E, Richards J H, Claassen V P, O'Dell R E, Ferrel M A 2011 Species specific plant-soil interactions influence plant distribution on serpentine soils. Plant Soil 342, 327-344.

Lee W G, Hewitt A E 1982 Soil changes associated with development of vegetation on an ultramafic scree, northwest Otago, New Zealand. J Royal Soc New Zealand 12, 229–242.

Lee W G 1992 The serpentinized areas of New Zealand, their structure and ecology. In The ecology of areas with serpentinized rocks, a world view. Ed. Roberts BA, Proctor J. pp 375-417. Kluwer, Dordrecht.

Liaw A, Wiener M 2002 Classification and regression by randomForest. Rnews 2, 18-22.

Massaccesi L, Benucci G M N, Gigliotti G, Cocco S, Corti G, Agnelli A (2015) Rhizosphere effect of three plant species of environment under pediglacial conditions (Majella Massif, central Italy). Soil Biol. Biochem. 89, 184-195.

Matthews J A, Whittaker R H 1987 Vegetation succession on the Storbreen glacier foreland, Jotunheimen, Norway: a review. Arct. Alp. Res. 19, 385-395.

Matthews J A 1992 The ecology of recently-deglaciated terrain. A geoecological approach to glacier forelands and primary succession. Cambridge University Press, Cambridge.

Mattirolo E, Novarese V, Franchi S, Stella A 1951 Carta Geologica d'Italia 1:100000, foglio 29. Istituto Geografico Militare, Firenze.

McCune B, Leon D 2002 Equations for potential annual direct incident radiation and heat load. J. Veg. Sci. 13, 603-606.

Mercalli L 2003 Atlante climatico della Valle d'Aosta. Società Meteorologica Italiana, Torino.

Moore A J, Kadereit J W 2013 The evolution of substrate differentiation in Minuartia series Laricifoliae (Caryophyllaceae) in the European Alps: In situ origin or repeated colonization? Am. J. Bot.100(12), 2412-2425.

Nagy L, Proctor J 1997a Soil Mg and Ni as a causal factors of plant occurrence and distribution at the Meikle Kilrannoch ultramafic site in Scotland. New Phytol. 135, 561-566.

Nagy L, Proctor J 1997b Plant growth and reproduction on toxic Alpine ultramafic soils: adaptation to nutrient limitation. New Phytol. 137, 267-274.

Oksanen J, Blanchet F G, Kindt R, Legendre P, O'Hara R B, Simpson G L, Solymos P, Stevens M H H, Wagner H 2011 vegan: Community Ecology Package. R Package Version 2.0-0. http://CRAN.Rproject.org/package=vegan. Accessed 21 April 2013.

Pignatti S 1992 Flora d'Italia, vol 1–3. Edagricole, Bologna.

Porder S, Ramachandran S 2013 The phosphorus concentration of common rocks - a potential driver of ecosystem P status-. Plant Soil 367(1), 41-55.

Proctor J 1992 Chemical and ecological studies on the vegetation of ultramafic sites in Britain. . In The ecology of areas with serpentinized rocks, a world view. Ed. Roberts BA, Proctor J. pp 135-167. Kluwer, Dordrecht.

Proctor J 1997 Recent work on the ultramafic vegetation of Scotland. Bot. J. Scot. 49, 277-285.

Raffl C, Mallaun M, Mayer R, Erschbamer B 2006 Vegetation succession pattern and diversity changes in a glacier valley, central Alps, Austria. Arc., Antarct. Alp. Res. 38(3), 421-428.

Rajakaruna N, Boyd R 2008 The edaphic factor. In Encyclopaedia of Ecology. Vol. 2. Ed. S E Jorgensen SE, F B Fath B, pp. 1201–1207.

Richard J L 1985 Observations sur la sociologie et l'écologie de Carex fimbriata Schkuhr dans les Alpes. Bot. Helv. 95(2), 157-164.

Righi D, Huber K, Keller C 1999 Clay formation and Podzol development from postglacial moraines in Switzerland. Clay Min. 34, 319–322.

Robinson B H, Brooks R R, Kirkman J H, Gregg P E H, Gremigni P 1996 Plant-available elements in soils and their influence on the vegetation over ultramafic ("serpentine") rocks in New Zealand. J Royal Soc. New Zealand 26(4), 457–468.

Schimmelpfennig I, Schaefer J M, Akçar N, Koffman T, Ivy-ochs S, Schwartz R, Finkel R C, Zimmermann T 2014 A chronology of Holocene and Little Ice Age glacier culminations on the Steingletscher, Central Alps, Switzerland, based on high-sensitivity beryllium-10 moraine dating. Earth Plan. Sci. Lett. 393, 220-230.

Temme A J A M, Keckmann T, Harlaar P 2016 Silent play in a loud theatre - Dominantly time-dependent soil development in the geomorphologically active proglacial area of the Gepatsch glacier, Austria. Catena 147, 40-50.

Ter Braak C J F 1987 Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.

Tsiripidis I, Papaioannou A, Sapounidis V, Bergmeier E 2010 Approaching the serpentine factor at a local scale – a study in an ultramafic area in northern Greece. Plant Soil 329, 35-50.

Ugolini F C 1966 Part 3. Soils. In: Mirskey A (Ed) Soil development and ecological succession in a deglaciated area of Muir Inlet, southeast Alaska. Institute of Polar Studies report Number 20, Ohio State University, Columbus, USA.

van Reeuwijk L P 2002 Procedures for Soil Analysis. Technical Paper n. 9. International Soil Reference and Information Centre, Wageningen, Netherlands.

Fig. 1 The study area in the North-western Italian Alps

Fig. 2 boxplots showing median values, upper and lower quartiles, upper and lower hinges (values as high as 1.5 times the quartiles) and outliers, and significant differences in the main chemical properties between eastern (E), central (C) and western sites (W), obtained from one-way ANOVA analysis, tukey test. From left to right, up to down: Nit, Pt, Ni, log transformed P, log transformed Ca, Log transformed Mg, Ca/Mg molar ratio, species richness, Ni/Nit ratio.

Fig. 3 Qualitative chronofunctions of Pt, Ni and Nsp (species richness) along the three different chronosequences in the Verra Grande forefield. P-values are shown for significant regression lines.

Fig. 4 Cluster dendrogram and the distribution of plant communities in the Verra Grande forefield. Dotted lines evidence the different LIA moraine systems.

Fig. 5 age distribution, and number of the clusters in the three moraine sectors of the Verra Grande Glacier; the number of species is in brackets.

Fig. 6 CCA scatterplot of plant communities along soil-environmental gradients; the studied sites are shown by their cluster number.

Fig. 7 Boxplot showing possible bioaccumulation or leaching indicators (respectively, Ca/Mg, P/Pt and Ni/Nit) under well vegetated plant communities (clusters 2, 5, 6, 7, 8, 9, 10); significant differences obtained from one-way ANOVA analysis are evidenced by the small letters above.

Figure 1

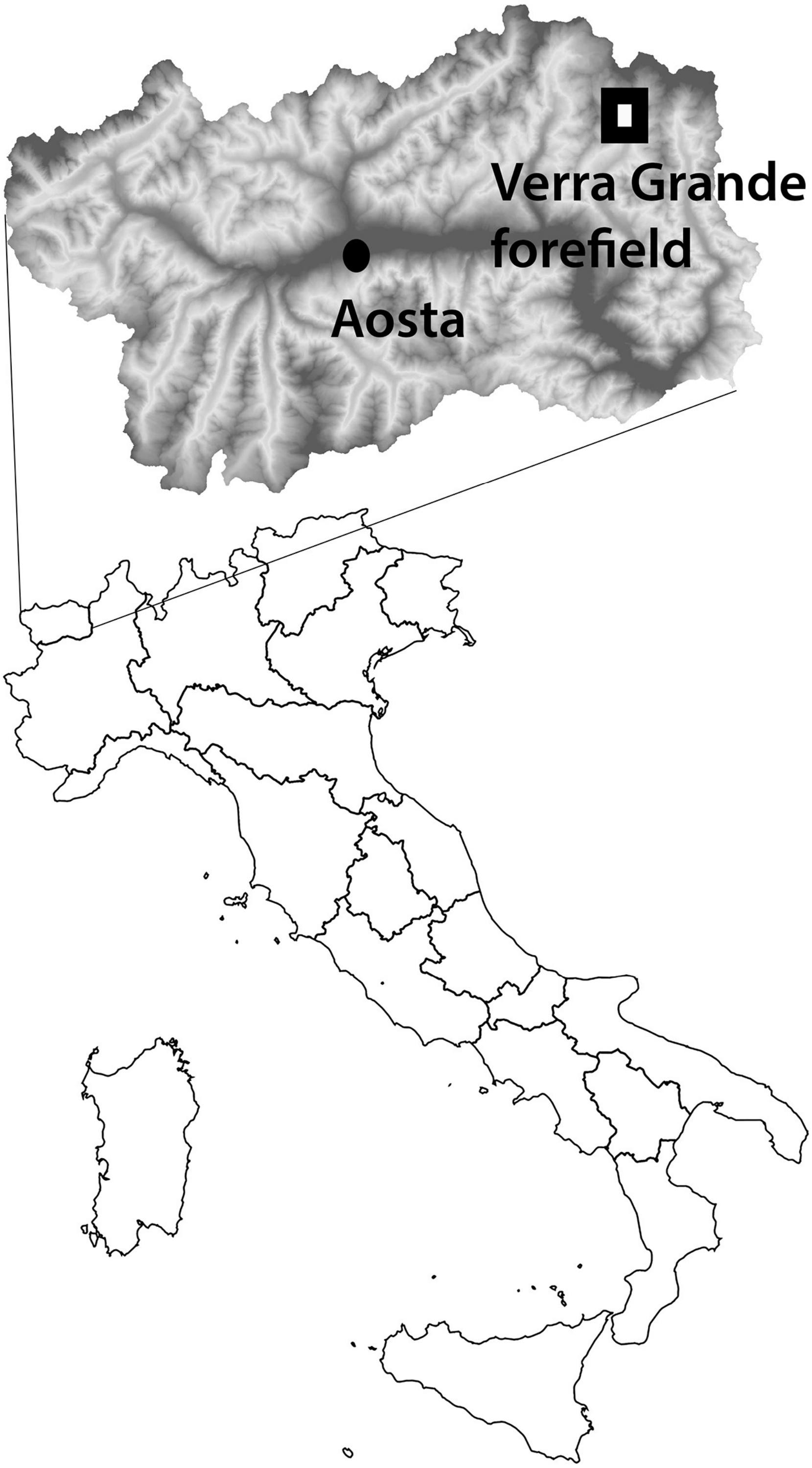
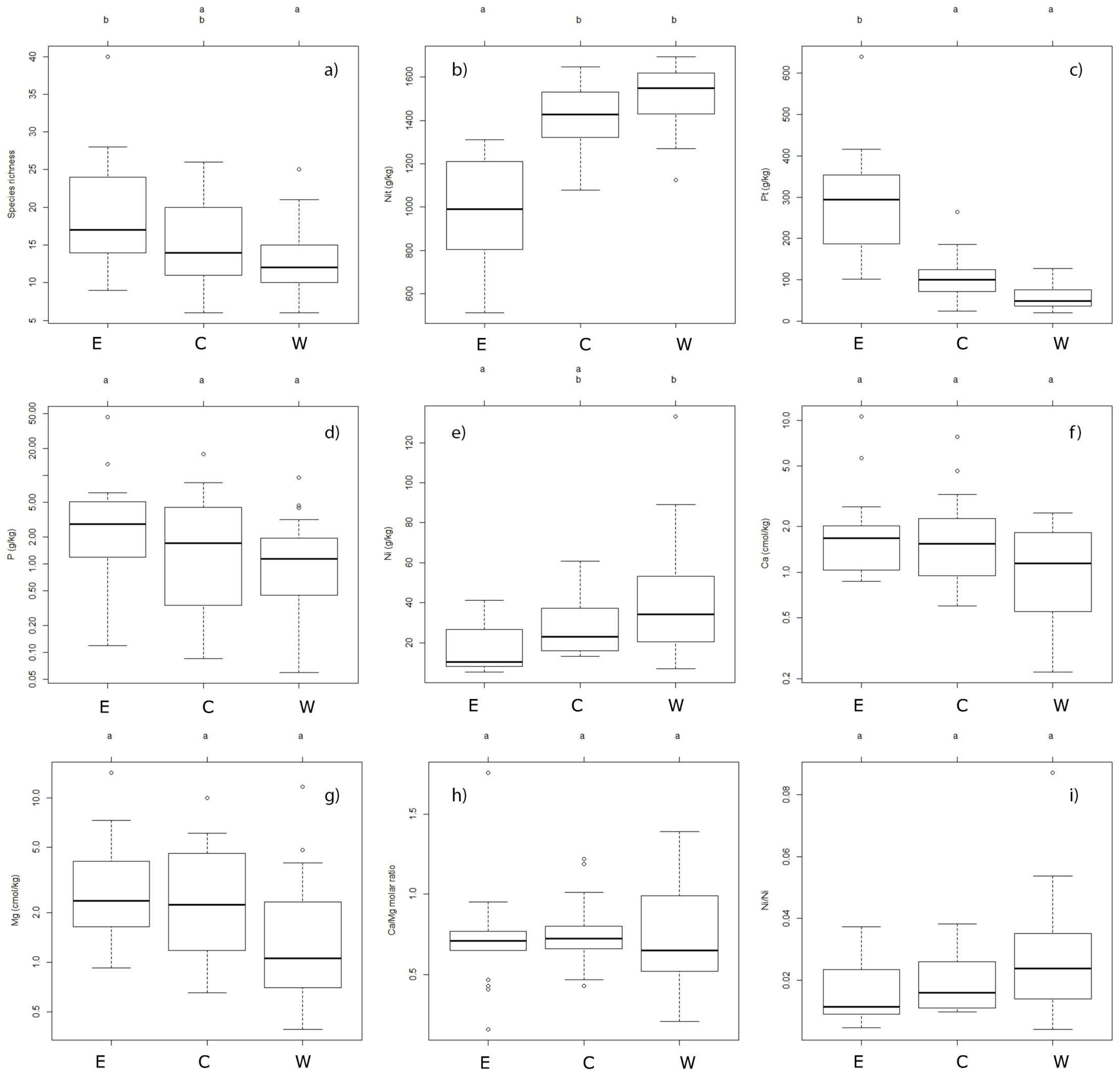


Figure 2



a)
Species richness
b)
Nit (g/kg)
c)
Pt (g/kg)
d)
P (g/kg)
e)
Ni (g/kg)
f)
Ca (cmol/kg)
g)
Mg (cmol/kg)
h)
Ca/Mg molar ratio
i)
Ni/Nt
E
C
W

Figure 3

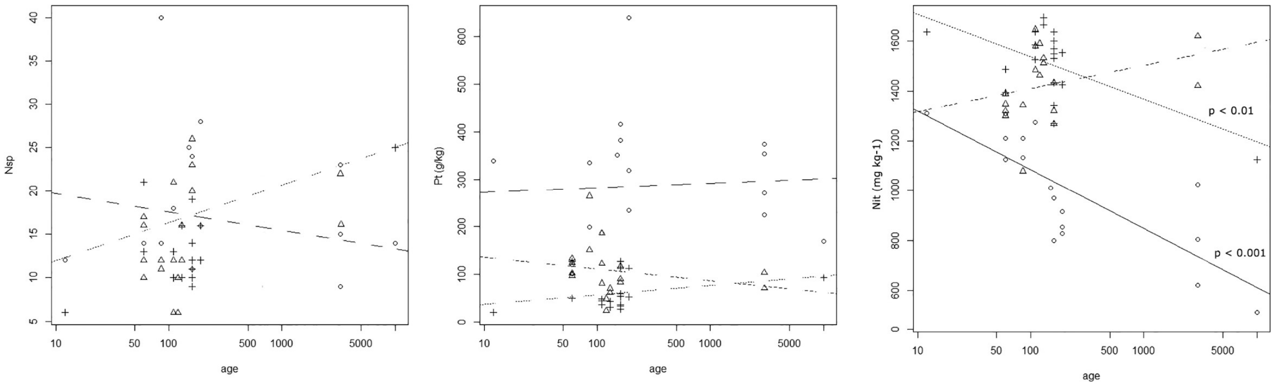


Figure 4

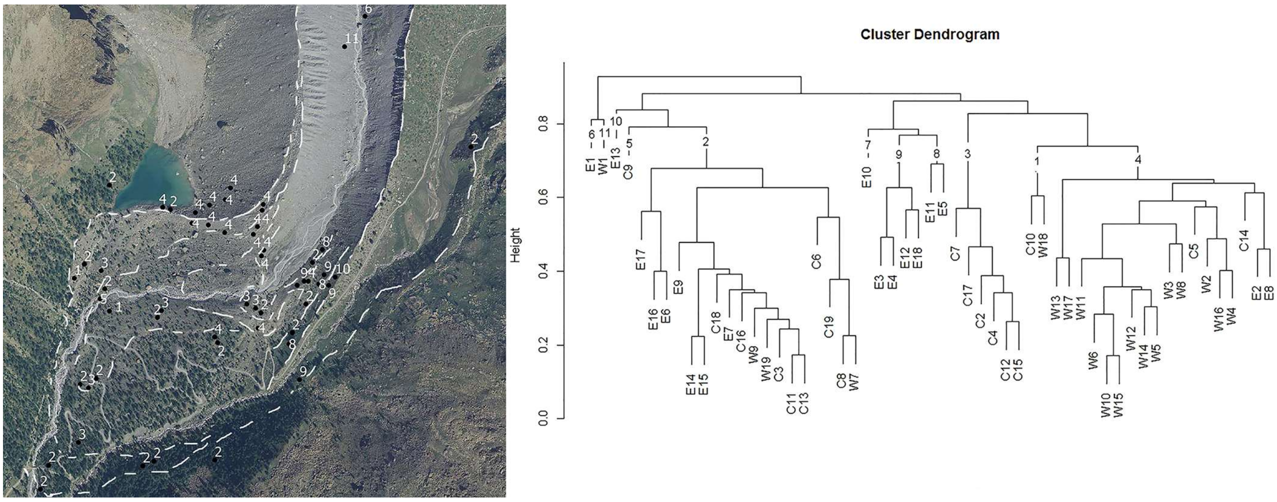


Figure 5

		Age since stabilization (year)						
		5 (2010)	70 (1945)	95 (1920)	155 (1860)	190 (1820)	700-3000 BP	11500 BP
	clusters	Number of sites (average number of species)						
	2		1 (14)	1 (18)		1 (16)	3 (39)	1 (14)
	10					1 (28)		
	8				1 (11)	1 (28)		
	9			1 (40)	2 (25)		1 (23)	
	7			1 (14)				
	4		2 (18)					
East	6	1 (12)						
	2		1 (12)	3 (7)	1 (16)	1 (20)	2 (19)	
	5			1 (21)				
	1			1 (12)				
	3		2 (14)	2 (11)	2 (25)			
Centre	4		1 (16)		1 (12)			
	2				1 (9)	1 (12)		1 (25)
	1					1 (16)		
	4		2 (17)	4 12)	6 (12)	2 (17)		
West	11	1 (6)						

Figure 6

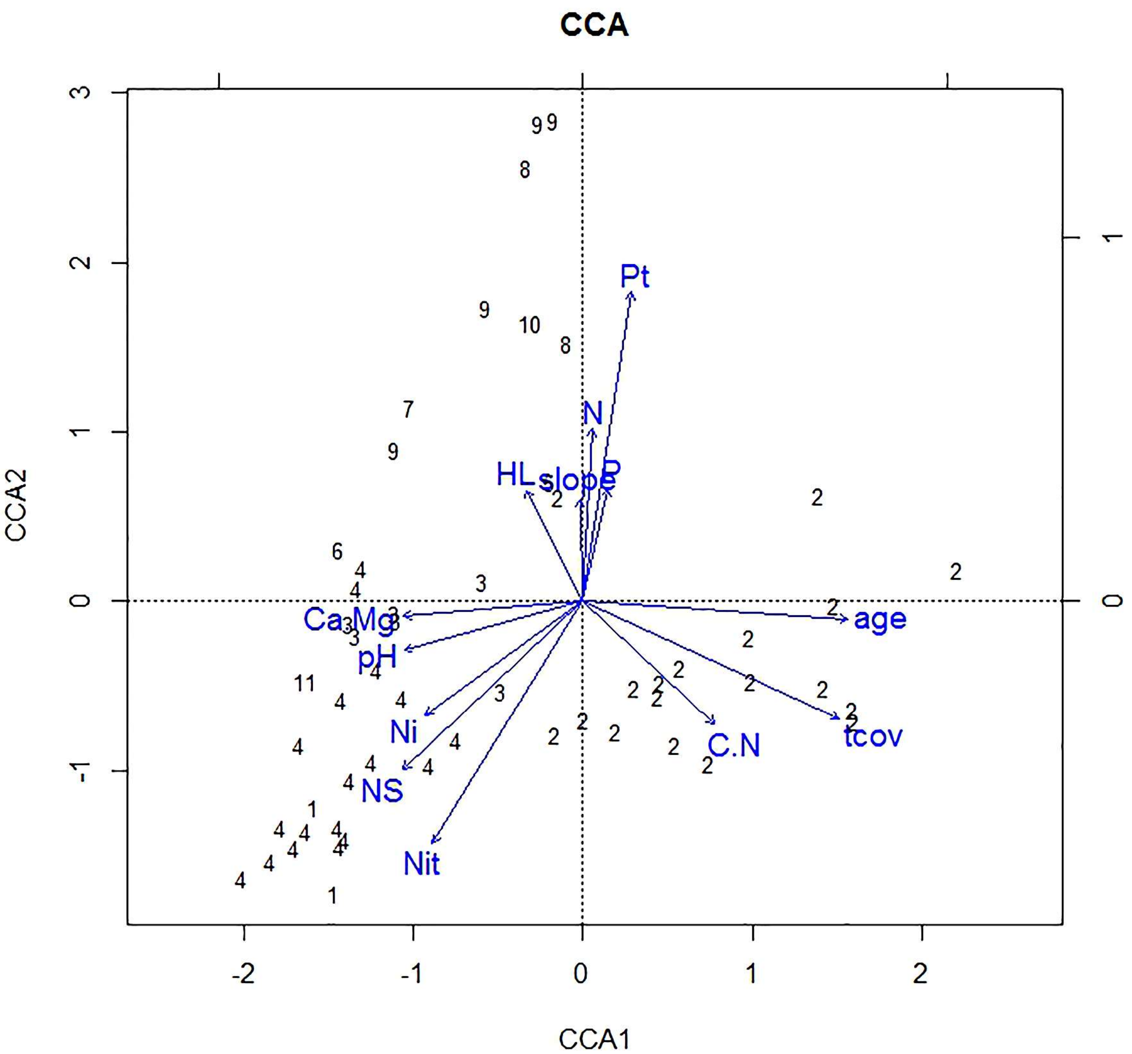


Figure 7

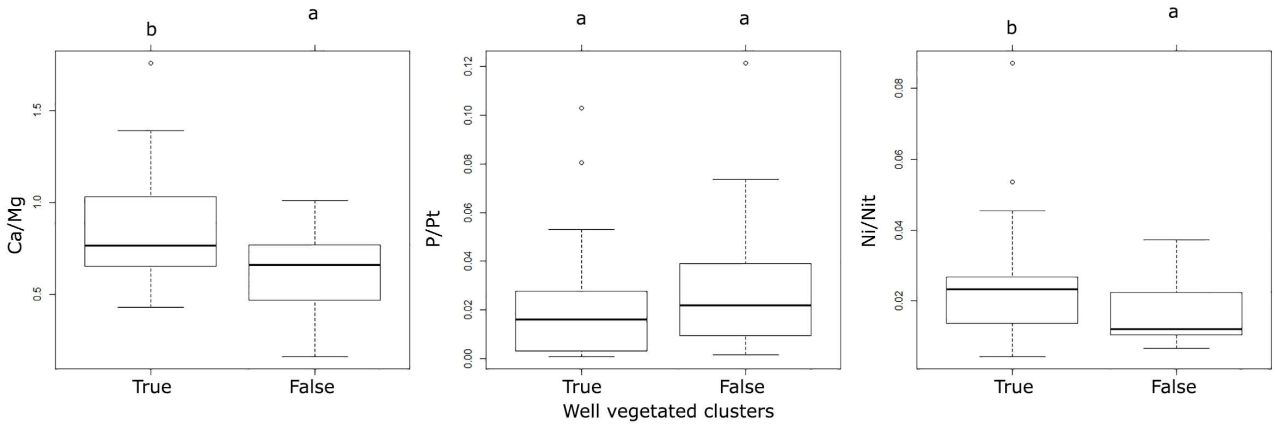

b
a
Ca/Mg
1.5
1.0
0.5
True
False
a
a
P/Pt
0.12
0.10
0.08
0.06
0.04
0.02
0.00
True
False
Well vegetated clusters
b
a
Ni/Nit
0.08
0.06
0.04
0.02
True
False

Table 1: eigenvalues, variance explaining factors, scores and significance (-: p value<0.1; *: p value < 0.05; **: p value < 0.01; ***: p value < 0.001) of the selected independent CCA variables. Soil-environmental factors are shown in order of importance, obtained through a stepwise analysis based on AIC statistics (function ordistep in the vegan package, Oksanen et al., 2011)

	CCA1	CCA2	CCA3	
Eigenvalue	0.522	0.369	0.293	Pr(>F)
Variance explained	0.089	0.063	0.050	
age	0.726	-0.049	-0.439	***
tcov	0.702	-0.321	0.465	***
Slope	-0.008	0.280	-0.134	**
Nit	-0.413	-0.666	-0.080	**
N	0.026	0.473	-0.151	-
P	0.069	0.309	0.008	*
Pt	0.134	0.849	0.065	*
HL	-0.157	-0.350	-0.350	*
C/N	0.361	-0.337	0.233	*
NS	-0.494	-0.463	0.065	
pH	-0.491	-0.134	0.128	
Ca/Mg	-0.492	-0.040	-0.182	
Ni	-0.434	-0.312	-0.197	-

Table 2: Random Forest rankings of the main environmental and edaphic factors on vegetation communities (derived from the cluster analysis) and on common species. The numbers represent positive (+), negative (-) or intermediate (+-, meaning positive correlation at low values, negative at higher ones) correlations on an arbitrary scale of decreasing importance of the factor in the presence/absence of the species (i.e., 1 is the most important factor, 7 the least); the correlation directions were obtained with GAM models. Only species growing in more than 5 or more of the sampling plots were considered. The results for the largest vegetation clusters are shown only if there are significant factors. Endemic species are indicated by *.

	pH	Ca	Mg	Ca/Mg	Ni	TOC	C/N	P	Age	slope	HL	Tcov	NS	N	Nitot	Ptot
Bare communities *		5-	3-	7+-	6+			4-							2+	1-
Well vegetated communities **	7-	6+	3+	9-	8-			5+	4+						2-	1+
Eastern sector					3-			4+							1-	2+
Western sector		4-	5-									3-			2+	1-
Achillea atrata											2+				1-	
Anthyllis vulneraria s.l.		4+-					5-		1-						2-	3+
Anthoxanthum alpinum	2-	4+										3+			1-	
Campanula cochelariifolia		4-	5-	6+	3+		2-	4(+)-	1-							
Campanula scheuchzeri					1-			4+				3+			2-	
*Cardamine plumieri**		7-			3+		5-	8-		6+		1-	2+		4+	
Carduus defloratus		4+-	2+-				3-							1+		
*Carex fimbriata**	1-			4-	5+					2-						3-
Carex sempervirens	1-		4+						2+			5+			6-	3+
Cerastium arvense														2+	1-	
Dryas octopetala					5+-		1+-		4-	2-	3+					
Epilobium fleischeri	4+								1-			3-	2+			
Festuca varia	4-	3+	2+		5-				1+							
Hieracium murorum s.l.	3-		4+-					5+	2+			6+	1-			
Homogyne alpina	5-				4-			3+-	1+						6-	

Juniperus communis	2-								1+							
Larix decidua			2+	3-							2-		4-			
Leonthodon helveticus	3-					2+						1-	4-			
Leucanthemum vulgare		5+	4+				1-	6+					2-	3+		
Linaria alpina		5-	4-	6+		1-							3+	2-		
Lotus corniculatus subsp. *alpinus*												1+				
Luzula lutea			4-		1+								2+--		5+	3-
Minuartia laricifolia	4+-				5+	2+	3+		1-					6+-		
Minuartia verna			2-		4+			3+-	5-			1				
Orthilia secunda			3+-		6-			4+		7-	2-	1+			5-	8+
Poa alpina			3+						4+-						2+-	1+
Poa minor	4+		3-				2-		1-							
Rhinanthus alectorolophus			7+		6-		4-				5+		2-		1-	3+
Rhododendron ferrugineum	2-		4+-	6-					1+			3+	5-			
Salix helvetica		1-	5-					3-						4-	2+-	
Salix breviserrata	4+				2+				1-				-		3+	
Saxifraga oppositifolia					1+											
Saxifraga paniculata					1+				4-			2-			3+	
Sempervivum arachnoideum				2+-	3+							1-				
Sempervivum montanum					4+	3-		1-						2-		
Silene vulgaris s.l.		2-	5-			4-				1+	3-			6-		
Solidago virgaurea s.l.		3+							2+					1+		
*Thlaspi sylvium**	1-		6+		5+	2+			4+-				7-	3+		
Thymus serpyllum							2-					3+-		5+	4+-	1+
Trichophorum caespitosum						2+								1+		
Trifolium pallescens	2+				4+-				3+-						1+-	
Trifolium pratense			2+		7-	6+	3-				5+		1-	4+		

Trisetum distichophyllum	6+	2-			4+				1-			3-			5+	
Vaccinium myrtillus									1+			3+	2-		4-	

*: clusters 1, 3, 4, 11

**: clusters 2, 5, 6, 7, 8, 9, 10