Changes in Community Composition Following Secondary Succession of a Temperate Oak-hornbeam Forest After Grassland Abandonment and Their Effect on Soil Carbon and Nutrient Pools

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Research

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Abstract

To date, changes in C and nutrient cycling during succession are somewhat studied, however, results are often contrasting for different nutrients and successional sequences. Generally, due to increment of litter rich in lignocellulosic components during late succession, mineralization of nutrients is expected to decelerate, and large amounts of nutrients become captivated within tree biomass. We investigated the changes in community composition following secondary succession of oak-hornbeam forest after grassland abandonment, along with the differences in soil chemistry between early- and late-successional stages. We aimed to discover whether late succession increases soil organic C and total N and S pool, but decreases the pools of plant available P and K, and that of micronutrients due to their captivation within the tree biomass.

The successional sequence studied had a following pathway: Helictotrichon pubescens haypastures à Brachypodium pinnatum successional grasslands à Cornus sanguinea scrubs à late-successional Populus tremula forests à late-successional oak-hornbeam (Quercus-Carpinus) forests. Total species number was highest in haypastures and lowest in late-successional P tremula forest. Species richness of haypastures was higher from that of mid-successional scrub and late-successional forest stages. Species richness did not differ between mid-successional scrub and late-successional forest stages. Occurrences of plant species throughout secondary succession was mostly stage-specific; only Fragaria vesca, Ajuga reptans, Cornus sanguinea, Prunus spinosa, and Viola hirta showed survival ability throughout almost all stages. Late-successional forest stages had a higher soil organic carbon (SOC), soil organic matter (SOM), and K\textsubscript{A} concentration, along with the higher soil C:N and C:S ratio in A horizon compared to early-successional grassland stages, whereas concentrations of plant available P and total N, S, Mn, Zn, Ni, Cu, and Fe remained unaltered.

Even though late-successional forest stages tighten the nutrient cycles through nutrient captivation within the tree biomass, we found that late succession efficiently retained P\textsubscript{A} and micronutrient pools and even increased K\textsubscript{A} concentration in the A horizon, despite the fact that great amounts of these nutrients were already excluded from the nutrient cycle. Despite the contradicting C inputs, soil total N and S concentration did not differ between late-successional forest and early-successional grassland stages.

Introduction

In the last few decades, changes in soil chemistry and nutrient cycling following both primary and secondary succession have been well studied; however, the available data is still insufficient to identify general patterns of how different successional stages alter soil chemical properties, especially soil nutrient pools. Effort has been made to postulate general rules that describe parallel changes in gross and net primary production during ecosystem development with soil nutrient cycling (Odum, 1969; Vitousek, 1975; Robertson and Vitousek, 1981), but the main limitation of these rules is that they can differ across biomes, vegetation sequences, soil types, and land use histories of the terrain on which succession is studied. Odum (1969) formulated 24 hypothesis of ecosystem development, three of which
were directly linked to nutrient cycling, and since then, these three hypotheses were tested several times, yielding somewhat mixed results.

To date, most of the effort in describing the links between vegetation succession and nutrient cycling has been made regarding N cycling, and somewhat less regarding P cycling (Robertson and Vitousek, 1981; Zak et al., 1990; Huang et al., 2013; Zhang et al., 2015), because these two macronutrients are typically the most limiting in ecosystem development. On the other hand, not many studies have investigated the cycling of K, Ca, and Mg (Bautista-Cruz and del Castillo, 2005; Feldpausch et al., 2004), and to our knowledge, no studies have focused on the cycling of micronutrients during vegetation succession.

Due to the different functional composition between most grassland and forest communities, it can be expected that these two vegetation types exert a different influence on nutrient retention and cycling. Plants in most early successional stages (except those growing on very nutrient-poor soils) are often characterized by lower C:N and C:P ratios, higher N and P contents, and lower content of lignocellulosic components in their tissues than those of species from late-successional stages, such as forests (Cortez et al., 2007; Poorter et al., 2004; Vitousek et al., 1988). Therefore, it can be expected that litter mineralization would be much faster in early-successional grasslands than in late-successional stages, as the high C content (especially lignin content) in the tissues of late-successional tree species delays the release of nutrient forms available to plants. For example, the release of nutrients from woody litter sometimes begins after as much as 60 years (Lambert et al., 1980). In addition, large amounts of nutrients are locked within the tree biomass and excluded from the nutrient cycle, which is why except in very fertile soils, the further supply of soil nutrients in forests often mainly depends on the amounts of nutrients available from parent material weathering, litter mineralization, or atmospheric deposition in the case of N and S in polluted areas (Vitousek et al., 1988). Furthermore, when mineralized, nutrients in forest soils are expected to be retained in the soil for only a short time because of their fast acquisition (i.e. recycling) by trees; for this reason, Odum (1969) suggested that nutrient cycles in late succession (i.e. forest stages) should be called “closed cycles”. Despite the general differences in the abovementioned functional traits between early and late-successional species, the results of previous studies on the influence of succession on soil nutrient pools are still somewhat unclear.

Changes in plant community composition following both primary and secondary succession have been somewhat studied on a global scale. However, these changes are biome-specific, and they can even differ within a single study area of the same climate and same climax community. This can make it hard to formulate some generalizations about floristic changes during succession, except in the cases when generalizations are made for a specific area of interest. In Croatia, vegetation succession is poorly studied. Only rare data is available on grassland succession (Kutnjak, 2010; Krstonošić et al., 2016), whereas somewhat more comprehensive research on the whole successional sequences has been carried out in the neighboring country of Slovenia (Čarni et al., 2007; Čarni et al., 2020; Dakskobler, 2010).

The aims of the present study were: (i) to examine the changes in community composition following secondary succession of temperate oak-hornbeam forests after grassland abandonment in NW Croatia;
(ii) to assess whether late succession (i.e. forest stages) decreases the concentrations of \( P_A \), \( K_A \), and micronutrients in the A horizon as a consequence of their captivation within the tree biomass, but increases soil total N and S concentrations, as well as organic C and organic matter content in contrast to those in early succession (i.e. grassland stages). Species nomenclature in this study followed The Plant List version 1.01 (2013).

**Materials And Methods**

**2.1. Study site**

The study was conducted in NW Croatia, in the surroundings of the village Brlog Ozaljski (45° 37' 32.37" N, 15° 24' 11.09" E). The mean annual rainfall of the study site ranges between 1000 and 1200 mm, mean annual temperature is 9–10°C, and the mean temperatures of the coldest (January) and warmest month (July) range from -2 to -1°C and from 19 to 20°C, respectively (Zaninović et al., 2008). The soil type the study was conducted on was slightly leached (i.e. leisivated) calcocambisol (Husnjak, 2014; Bogunović, 1998) on biolithitic and bioclastic limestones (Bukovac, 1984). In WRB classification (2006), this soil type corresponds to calcic cambisol. This is the dominant soil type in the study area, with district cambisol on quaternary quartz sands, gravels, and red siltose and grey clays as inclusions (Bukovac, 1984; Bogunović, 1998). In the study area, during the last few decades, the abandonment of agricultural land use led to a progression of vegetation succession, resulting in significant loss of meadow and pasture communities. Today, the remaining meadows are dominated by grass species *Helictotrichon pubescens* and are used as haypastures, i.e. for obtaining hay, but are also occasionally used for low-intensity rotational grazing by sheep.

**2.2. Vegetation survey and soil sampling**

The sequence of vegetation succession was studied using the space for time substitution, and five stages of succession were included in the study (Fig. 1). The investigated successional stages were selected to be near one another as much as possible and in a relief conditions as similar as possible in order to exclude the influence of differing slope and exposition. The vegetation survey was performed throughout May, June, July, and August 2020, using five 100 m\(^2\) plots (10 × 10 m) within each successional stage, totaling 25 plots. Species cover in grasslands was recorded using the phytosociological scale of Barkman et al. (1964), and in scrub and forest stages using the scale of Braun-Blanquet (1964).

For the analyses of differences in soil pH, SOC, and nutrient concentrations between the two end-points of succession, six pairs of early-successional grassland and late-successional forest stages located next to each other were selected. Every pair of grassland and forest plots represented a block (i.e. randomized block design). The grasslands selected for this purpose were recently used haypastures undergoing colonization with successional grasses, whereas forest plots selected for this purpose were late-successional mixed stands of *P. tremula*, *C. betulus*, and *Q. petraea* and/or *Q. robur* (see the footnotes in
Table A1). Both grassland and forest plots selected for the soil analyses belonged to the same chronosequence used to study the changes in community composition. Soil was sampled at the depth of 0–10 cm, which corresponded to the A horizon. Soil sampling was performed from February 28 to March 2, 2020, before the beginning of the vegetation season. This time of the year was chosen for soil sampling because most of the nutrients were still retained in the soil and were not yet acquired by vegetation; thus, we wanted to examine their statuses in the surface soil before uptake by vegetation. Litter leftovers were removed from the soil samples. The soil was not sampled from the scrub stage of *C. sanguinea* as it is a mid-successional stage.

### 2.3. Soil laboratory analyses

Soil analyses were carried out using air-dried, homogenized, and sieved soil samples (<2 mm sieve) (prepared according to the protocol ISO 11464). We determined the soil pH in 1:2.5 (w/v) soil suspension in 1 M KCl (modified ISO 10390). Organic C, and total N and S concentrations were determined by the dry combustion method (HRN ISO 10694:2004, HRN ISO 13878:2004, and HRN ISO 15178:2005, respectively). Soil organic matter content was calculated according to Škorić (1982). Plant available P and K were extracted with an ammonium lactate (AL) solution (Egner et al., 1960) and their concentrations were detected by spectrophotometry and ame photometry, respectively. Total concentrations of Mn, Zn, Fe, Cu, and Ni were determined using the pXRF method with an Olympus-Vanta C series portable X-ray fluorescence analyzer (HRN EN ISO 13196:2015).

### 2.4. Statistical analyses

Differences in community composition between the successional stages were plotted with non-metric multidimensional scaling (NMDS) using a dissimilarity matrix constructed with Bray-Curtis dissimilarities. Prior to the NMDS, cover values of species occurring in more than one vegetation layer were pooled, and all of the cover values were transformed to type I ordinal percentage scale (Van der Maarel, 2007). Differences in mean species richness between the successional stages were analyzed by one-way ANOVA, followed by Tukey’s post-hoc pairwise comparison test.

The differences in soil chemistry between early and late succession were analyzed by two-way ANOVA without replication in order to account for inter-site variation (i.e., the block effect). One-way ANOVA without blocking was used for the variables that were not significantly influenced by inter-site variation (i.e., if the block effect was >0.25). Threshold value of 0.25 was chosen for assessing the significance of the block effect because it is stricter than the traditional threshold of 0.05 for the purpose of determining the inter-site variation of the soil chemical properties. All data were analyzed using the PAST 4.03 software (Hammer et al., 2001).

### Results

#### 3.1. Differences in community composition following secondary succession
The NMDS ordination plot (Fig. 2) provided a very good representation of the community composition differences between the successional stages of the analyzed chronosequence, with a final stress for 2D solution of 11%. Early-successional stages (i.e. haypastures and B. pinnatum-dominated successional grasslands) were grouped close together and ordinated on the negative side of the first axis, whereas late-successional stages (i.e. P. tremula forests and oak-hornbeam forests) were ordinated on the positive side of the first axis, and C. sanguinea scrubs were ordinated in the middle between all other stages.

Figure 3 shows the differences in species richness (i.e. number of species per plot) between the different successional stages. Species richness of haypastures was significantly higher than that of mid-successional C. sanguinea scrubs, as well as that of late-successional P. tremula forests and oak-hornbeam forests. There were no significant differences in mean species richness between mid- and late-successional stages. Species richness of successional grasslands dominated by B. pinnatum significantly differed from that of P. tremula forests, but did not differ from that of C. sanguinea scrubs and oak-hornbeam forests. Species richness of different succession stages was as follows: haypastures had an average of 41 species per 100 m\(^2\); successional grasslands had an average of 32 species per 100 m\(^2\); C. sanguinea scrubs had an average of 26 species per 100 m\(^2\); late-successional P. tremula forests had an average of 20 species per 100 m\(^2\); and late-successional oak-hornbeam forests had an average of 24 species per 100 m\(^2\). Full results of Tukey pairwise comparison test are given in Table A3.

Total species number in haypastures, successional grasslands, C. sanguinea scrubs, P. tremula forests, and oak-hornbeam forests was 76, 72, 59, 46, and 59, respectively.

3.2. Floristic characteristics of individual succession stages

Plant community composition of individual successional stages within the analyzed chronosequence is listed in Table A1.

1. Haypastures (HP)

With the highest overall abundance, the dominant species of haypastures was the grass Helictotrichon pubescens. Among other grass species, Anthoxanthum odoratum and Trisetum avescens appeared in all five plots with somewhat high overall abundance, followed by Holcus lanatus appearing in four plots, also with high overall abundance, and Dactylis glomerata and Festuca ovina appearing in four plots with low abundance. Trifolium pratense was the most abundant legume, appearing in all five plots, followed by less abundant T. repens, appearing in four plots. Among Asteraceae, Leucanthemum vulgare, Achillea millefolium, and the invasive Erigeron annuus appeared in all five plots, followed by Leontodon hispidus appearing in four plots. Among Lamiaceae, only Clinopodium vulgare appeared in all five plots, and among Apiaceae, Pimpinella saxifraga was the most abundant species, also appearing in all five plots. Other frequent species in haypastures appearing in all five plots were Plantago lanceolata, Fragaria
vesca, and *Ranunculus bulbosus*, and those appearing in four plots were *Polygala comosa, Daucus carota, Galium mollugo, G. verum, Ranunculus acris, Rumex acetosa*, and *Veronica chamaedrys*.

2. Successional grasslands (SG)

In successional grasslands, the grass *Brachypodium pinnatum* was the dominant species. *Calamagrostis epigejos* appeared only in two plots, but was found in the study area forming patches within *B. pinnatum* grasslands. Other species occurring in all five plots were the grasses *Dactylis glomerata* and *Briza media*, along with the mid-successional species *Cornus sanguinea* and *Prunus spinosa* occurring in the herb layer. Species occurring in four plots were *Fragaria vesca, Filipendula vulgaris, Pastinaca sativa*, and *Veronica chamaedrys*.

3. *Cornus sanguinea* scrubs (CS)

*Cornus sanguinea* was the dominant species in the shrub layer of this stage, with a cover of 90–100% in all five plots. Almost all species in the herb layer had a cover of <1%. Other species that appeared in four or five plots were growing in the herb layer, and these were *Erigeron annuus, Centaurea jacea, Ajuga reptans, Clinopodium vulgare, Fragaria vesca, Veronica chamaedrys*, and *Rubus plicatus*, as well as seedlings of *C. sanguinea, Prunus spinosa*, and *Euonymus europaeus*.

4. Late-successional *Populus tremula* forest stage (PT)

This stage of late forest succession was characterized by the dominance of *Populus tremula* in the tree layer. In the shrub layer of this stage, *Cornus sanguinea* had a somewhat higher dominance than that of other species and occurred in all five plots, whereas *Corylus avellana* occurred in four plots, also with higher overall abundance than that of other shrub species. In the herb layer, almost all species had a cover <1%. The most frequent species in the herb layer were seedlings of *Carpinus betulus* and *Cornus sanguinea*, appearing in all five plots, and *Hedera helix*, also appearing in all five plots. These were followed by seedlings of *Prunus spinosa* and *Prunus avium*, occurring in four plots.

5. Late-successional oak-hornbeam forest stage (OH)

This was the last successional stage within the analyzed chronosequence, and it was closest to the terminal (i.e. climax) forest. This stage was dominated by *Carpinus betulus* in the tree layer. Besides *C. betulus, Quercus robur* appeared in the tree layer of three plots, and *Populus tremula* in the tree layer of two plots. In the shrub layer, only *Corylus avellana* appeared in four plots. In the herb layer, the species occurring in all five plots were *Epimedium alpinum* (with the highest abundance), *Rubus hirtus*, and seedlings of *C. betulus*. These were followed by *Ajuga reptans, Hedera helix, Primula vulgaris*, and seedlings of *Q. robur* and *Crataegus monogyna*, which all occurred in four plots.

3.3. Differences in soil chemistry between early and late succession
The results suggested that after grassland abandonment, the SOC and SOM contents in the A horizon increased by 16 g kg\(^{-1}\) and 28 g kg\(^{-1}\) on average, respectively (P<0.05), in the late-successional forest stages (Table 1 and Table A2). The forest stages also had a higher C:N ratio than that of grassland stages (P<0.0001), and a higher C:S ratio than that of grassland stages (P<0.05) in the A horizon, whereas soil total N and S concentrations and soil N:S ratio remained unaltered (P>0.05) (Table 1). The results also suggested that the concentration of P\(_A\) in the A horizon did not decrease in the forest stages compared to that in grassland stages. The late-successional forest stages also significantly increased the K\(_A\) concentration in the A horizon (P<0.05) compared to that of grassland stages, whereas the concentrations of total Mn, Zn, Cu, Ni, and Fe remained unaltered in forest A horizon (P>0.05) (Table 1). Detailed results of our soil laboratory analyses are given in Table A2.

**Discussion**

**4.1. How plant community composition changed during secondary succession**

Secondary succession in the analyzed chronosequence seemed to progress in the following manner. After the abandonment of haypastures, *Brachypodium pinnatum* completely colonized the habitat, reaching a cover of >75%, and as a consequence of this colonization, the cover of most subordinate species remained <1%. The abundance of grass species from haypastures, such as *Helictotrichon pubescens*, *Anthoxanthum odoratum*, and *Holcus lanatus*, slightly decreased in successional grasslands, whereas *Dactylis glomerata* and *Briza media* seemed to persist, and their occurrence increased in successional grasslands. The colonization of haypastures by *B. pinnatum* led to a decrease in the number of haypasture species, but did not induce their complete turnover. Haypastures, which are essentially meadow communities, had the highest species richness and highest total species number among all stages of succession. However, after their colonization by *B. pinnatum* and the consequential formation of successional grasslands, species richness did not significantly decrease (i.e. it only marginally decreased), and total species number was only slightly lower than that of haypastures. It seemed that some haypasture species from the study area had a stronger ability to cope with the spread of *B. pinnatum* than we expected.

Mid-successional shrub species *Cornus sanguinea* and *Prunus spinosa*, which later formed scrub stages in the study area, established their seedlings within the successional grasslands. During the mid-successional scrub stage of succession, *C. sanguinea* gained a complete dominance over the community, with a cover of 90–100%, and almost all species in the herb layer under its canopy persisted with a cover of <1%. Only *Rubus plicatus* occurred within the scrub stage with a somewhat high overall abundance because it was able to intertwine its stems around the branches of *C. sanguinea*, thus being able to acquire more sunlight for its growth. *Euonymus europaeus*, a mid-successional shrub species which was also frequent in the study area, began to occur with a somewhat high frequency during the scrub stage of succession; however, its cover did not reach high values within *C. sanguinea* scrubs. *Carpinus betulus*, a tree species specific for the last successional stage in the analyzed chronosequence, already appeared in the herb layer of the scrub stage in the form of seedlings under the dense canopy of *C. sanguinea*. 
Interestingly, the invasive *Erigeron annuus* was frequent in haypastures, ceased after *B. pinnatum* spread, and later appeared during the *C. sanguinea* scrub stage.

Late succession began with the development of the forest stage in which *Populus tremula* was the dominant species in the tree layer. *Betula pendula* was also found in the tree layer of this stage, and *C. betulus*, a tree species later dominant in the last successional stage, was already present in the tree layer of the *P. tremula* stage. *C. sanguinea* remained present in the shrub layer under the dense *P. tremula* canopy, but its dominance decreased compared to that in the previous scrub stage. In the shrub layer, *Corylus avellana*, a species that was not apparent in the previous stages of succession, seemed to have formed its bushes during the *P. tremula* stage. In the herb layer of this stage, *C. betulus* seedlings were frequent, and seedlings of *C. sanguinea* and *Prunus spinosa* still survived in these shaded conditions. *Hedera helix*, a species that is most often highly abundant in climax forests, was already frequent under the *P. tremula* stage, but with small abundance. In addition, *Epimedium alpinum*, a species also characteristic for climax forests, was already established in some of the plots during the *P. tremula* stage.

The last successional stage of the analyzed chronosequence was dominated by *C. betulus* in the tree layer, and *Quercus robur* was found besides it, but with lower frequency and abundance. This last stage was however only an approximate for true climax forests that were once present in the study area, but disappeared as a consequence of anthropogenic management (i.e. extraction of wood for fuel). This was also the reason why plots of 100 m$^2$ were adequate for the purposes of representing the forest stages in the study area, instead of the usual 400 m$^2$ plots which are used for investigating intact original forests. In this stage, *C. avellana* remained frequent in the shrub layer. *Rubus hirtus*, a species that only occasionally occurred in the previous successional stages, became frequent in the herb layer of the *C. betulus* forest stage. The dominant species in the herb layer under the *C. betulus* canopy was *E. alpinum*, which indicated that these late-successional forest stages are the most similar to those of potential climax forests in the study area.

The ability of species to survive within different successional stages throughout the investigated sequence differed. For example, species that showed an ability to survive through all stages were *Ajuga reptans* and *Fragaria vesca*. In addition, *Viola hirta* was not present in haypastures, but it appeared after the development of successional grasslands and persisted throughout the succession, including the last forest stage. The survival of *Veronica chamaedrys* was also uninterrupted from haypastures all the way to the *C. sanguinea* scrub stage, and a similar response was found for *Clinopodium vulgare*. In the herb layer, *C. sanguinea* and *P. spinosa* successfully grew all the way from the successional grassland stage to the late-successional *P. tremula* forest stage.

4.2. How secondary succession altered the soil chemical properties
Late-successional forest stages of the secondary succession after grassland abandonment increased the SOC and SOM contents in the A horizon, but the increase was not too great considering that the mean SOC and SOM contents in the early-successional grassland stages were 41 g kg\(^{-1}\) and 71 g kg\(^{-1}\), respectively, and that the in late-successional forest stages were 57 g kg\(^{-1}\) and 99 g kg\(^{-1}\), respectively. In addition, total soil N and S did not increase after the development of late-successional forest stages, even though this could be expected because of high organic matter content in forest soils which organic N and S originate from (Johnson, 1992; David et al., 1982). On the other hand, soil C:N and C:S ratios in the A horizon increased in late-successional forest stages compared to those in early-successional grassland stages. The changes in stoichiometric ratios of nutrients in soil, litter, and plant tissues that describe the nutrient limitation during succession are well studied (Jiao et al., 2013; Liu et al., 2020; Ouyang et al., 2017; Liang et al., 2017; Huan et al., 2017). Increased soil C:N ratio under forest stages usually indicates that N mineralization in these stages is somewhat slower than that in grasslands. Studies have suggested that N mineralization is slower in forest soils than in the soils of early successional stages, and that this is due to various processes related to inhibition of nitrification (Rice and Pancholy, 1972; Rice and Pancholy, 1973; Todd et al., 1975). However, the results of studies that compared N mineralization in forest stages and that in early successional stages are often mixed (Robertson and Vitousek, 1981; Vitousek et al., 1989; Yan et al., 2009), thus, a soil C:N ratio solely is not necessarily a safe measure for evaluating N mineralization rates.

In the present study, after grassland abandonment, late-successional forest stages greatly increased the \(K_A\) concentration in the A horizon. As the effects of secondary succession on the soil K pool are still understudied, it is hard to make a generalized conclusion about K cycling during succession. The increase in soil K pool following secondary succession has been reported in several studies (Feng et al., 2007; Wang et al., 2010; Wen et al., 2005; Liu and Huang 2005; Meng et al., 2010); however, available K and total K pools do not necessarily follow the same trend during succession. It is hard to conclude which exact reasons underlie the increased \(K_A\) concentration under forest stages in the present study. Either trees possess an ability to substitute \(K^+\) from the crystal lattice of clay minerals with \(H^+\), thus efficiently extracting it into the soil labile pool, or they are able to efficiently increase its concentration through leaf litter deposition. As the soil was sampled from the depth of \(\leq 10\) cm, our opinion is that both processes are likely to simultaneously contribute to the K supply at this soil depth. However, further studies on the plant nutritional status and litter decomposition rates during succession are required to clarify this.

On the other hand, late-successional forest stages neither increased nor decreased the \(P_A\), total Mn, Zn, Cu, Ni, and Fe concentration in the A horizon. This suggested that despite the fact that in forest ecosystems, most nutrients are locked within the tree biomass, late-successional forest stages in the present study were still able to efficiently retain \(P_A\) and micronutrients within their A horizons and inhibit the decline in their pools, thus saving them for their development during vegetation period. This is especially important for \(P_A\), which had a low concentration in the soils of both grassland and forest stages in the present study (ranging from 0.9 to 1.7 mg 100 g\(^{-1}\) in grasslands and from 1.0 to 2.9 mg 100 g\(^{-1}\) in forests), and because P is often the most limiting macronutrient in natural soils which practically
cannot be supplied from atmosphere (Vitousek et al., 1988). As trees require P for their growth and lock it inside their biomass, they remove large amounts of P from the nutrient cycle; thus, the further P supply of the forest soil depends either on P turnover by litter or further weathering of the parent material. Trees probably possess the ability to obtain P through exudation of organic acids originating from roots or from humus substances, which could enhance the dissolution of hardly soluble P (see Fox, 1995); however, it is also possible that in the present study, trees retained the P\textsubscript{A} in the A horizon through litter deposition.

The advantage of the present study was that it showed that on the same soil type influenced by the same pedogenic factors, late succession increased the SOC and SOM content, along with the plant available K pool, but it also retained the pools of P and micronutrients. The limitation of this study is that it only focused on the general differences in soil chemistry between early and late succession, but it did not account for these differences between every single stage of succession. Thus, further studies on this topic should focus on analyzing the differences in soil chemistry between all stages of succession, and try to link the observed changes with the dominant plant species within these stages.

From the perspective of vegetation ecology, this study showed the differences in community composition following secondary succession using phytosociological field methods, and it also showed which species survive in different stages of succession. These kind of studies are still rare in Europe as very hard effort is required to study such dense and hardly accessible mid-successional scrub stages of the phytosociological class 

\textit{Crataego-Prunetea Tx. 1962 nom. conserv. propos} (see Škvorc et al., 2017), which are only accessible by knee or army crawling. However, in order to better understand the community changes during the succession of the investigated oak-hornbeam forests in the future, a larger sample size is required, and all stages of succession should be studied using the scale of Barkman (1964), so that other measures of diversity could be assessed.

**Conclusions**

- Total species number generally decreased following secondary succession; as the initial stage of succession, haypastures had the highest total number of species, whereas late-successional \textit{Populus tremula} forest stage had the lowest total number of species.

- Species exhibiting the ability to survive through almost all five successional stages were \textit{Ajuga reptans}, \textit{Fragaria vesca}, \textit{Cornus sanguinea}, \textit{Prunus spinosa}, and \textit{Viola hirta}.

- \textit{Veronica chamaedrys}, \textit{Clinopodium vulgare}, \textit{Agrimonia eupatoria}, and (with lower frequency) \textit{Centaurea jacea} were present from the initial succession stage to the scrub stage, but mostly ceased after the development of late-successional forest stages. The occurrence of other species was mostly stage-specific.

- After grassland abandonment, the A horizon of late-successional forest stages had higher SOC and SOM content, plant available K concentration, and C:N and C:S ratios than those of the A horizon of early-successional grassland stages.
• The concentrations of plant available P and micronutrients did not decrease after the development of late-successional forest stages, indicating that their pools were efficiently retained in the forest soil despite the fact that their large amounts were already locked within the tree biomass and excluded from the nutrient cycle. Furthermore, despite the increased SOM content, total concentrations of N and S did not increase after the development of late-successional forest stages.

Declarations

Code availability

No custom made code was used in the preparation of this manuscript

Data availability

The data from this manuscript were not deposited in any online data repositories

Author contribution

Study conceptualization and manuscript preparation: A. J.; phytosociological survey: A. J. and D. P.; soil laboratory analyses: A. P. and Ž. Z.; editorial advice: D. P. and Ž. Z.

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**Table 1:**
The results of blocked ANOVA for the differences in soil chemical properties between early succession (i.e. grassland stages) and late succession (i.e. forest stages) (n=6)

| Soil variable     | F      | Block effect | Succession effect |
|-------------------|--------|--------------|-------------------|
| pH 1M KCl         | 0.0003 | 0.19         | 0.99              |
| SOC, g kg⁻¹       | 8.28   | 0.81         | 0.02              |
| SOM, g kg⁻¹       | 8.28   | 0.80         | 0.02              |
| N, g kg⁻¹         | 0.29   | 0.84         | 0.61              |
| S, g kg⁻¹         | 1.17   | 0.55         | 0.30              |
| C:N               | 296    | 0.03         | <0.0001           |
| C:S               | 7.32   | 0.50         | 0.02              |
| N:S               | 0.20   | 0.80         | 0.67              |
| Pₐ, mg 100 g⁻¹    | 2.28   | 0.57         | 0.19              |
| Kₐ, mg 100 g⁻¹    | 7.41   | 0.01         | 0.04              |
| Mn, mg kg⁻¹       | 0.11   | 0.35         | 0.75              |
| Zn, mg kg⁻¹       | 0.55   | 0.49         | 0.48              |
| Cu, mg kg⁻¹       | 0.21   | 0.75         | 0.66              |
| Ni, mg kg⁻¹       | 0.99   | 0.16         | 0.36              |
| Fe, g kg⁻¹        | 1.49   | 0.49         | 0.25              |
If the block effect was insignificant (P>0.25), then the F and P values for the succession effect corresponded to those of simple one-way ANOVA, whereas if the block effect was significant (P<0.25), then the F and P values for the succession effect corresponded to that of blocked ANOVA (i.e. two-way ANOVA without replication); SOC – soil organic carbon, SOM – soil organic matter, N – soil total nitrogen, S – soil total sulfur, C:N – soil carbon to nitrogen ratio, C:S – soil carbon to sulfur ratio, N:S – soil nitrogen to sulfur ratio, PA and KA – ammonium lactate-extracted plant available phosphorus and plant available potassium, respectively, Mn – soil total manganese, Zn – soil total zinc, Cu – soil total copper, Ni – soil total nickel, Fe – soil total iron.

**Figures**

**Figure 1**

Different successional pathways of forest development apparent within the study area (the sequence marked in green was analyzed in the present study). Legend: Helictotrichon pubescens haypasture – grasslands used as meadows, which also experience occasional rotational grazing by sheep; Brachypodium pinnatum successional grassland – successional grasslands dominated by Brachypodium pinnatum with patches of Calamagrostis epigejos; Pteridium aquilinum stage – stands of P. aquilinum; Cornus sanguinea scrub stage – mid-succession dominated by C. sanguinea; Prunus spinosa scrub stage – mid-succession dominated by P. spinosa; mixed Prunus-Cornus scrub stage – mid-
succession with both P. spinosa and C. sanguinea as dominant shrubs; Populus tremula forest stage – late-successional forest stage with P. tremula as the dominant tree species, with Betula pendula and Corylus avellana as accompanying species; Pteridium-Betula forest stage – late-successional forest stage with the fern P. aquilinum as the dominant species in the herb layer and B. pendula as the dominant species in the tree layer; Quercus-Carpinus forest stage – late-successional forest stage dominated by Carpinus betulus in the tree layer accompanied by Quercus robur and occasionally Quercus petraea.

Figure 2

Non-metric multidimensional scaling ordination of successional stages based on community composition differences, obtained by Bray-Curtis dissimilarities. The plot in the upper right corner represents the Shepard plot.
Figure 3

a) Differences in species richness (i.e. species number per unit area, in this case 100 m²) between the different successional stages within the single chronosequence (n=5). Black dots indicate the mean values, and different lowercase letters indicate significant differences at P < 0.01 according to Tukey’s pairwise comparison test; b) total species number recorded in different successional stages; HP – haypasture plots, SG – successional grassland plots, CS – Cornus sanguinea scrub plots, PT – Populus tremula forest plots, OH – oak-hornbeam forest plots

Supplementary Files

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