



Variations in soil chemical properties, microbial biomass, and faunal populations as related to plant functional traits, patch types, and successional stages at Sokolov post-mining site - A case study



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ARTICLE INFO

Handling editor: Thibaud Decaens

Keywords:

Post-mining sites
Chronosequence
Plant functional trait
Vegetation patch
Faunal surface activity

ABSTRACT

The majority of studies that investigate plant functional trait effects on ecosystem properties use traits obtained from databases. However, we know little regarding their explanatory power proportionate to that of the semi-quantitative vegetation properties, and much less still is known when considering their temporal changes in a chronosequence. We determined whether soil chemical and biological properties were associated with plant functional traits, observed vegetation properties (“patch type” = bare soil, grassy, woody), and site age (10, 18, 28, and 55 years), at four spontaneously overgrown post-mining locations in the Czech Republic. Abiotic soil properties were expected to be closely related to the site age, while above and below ground macrofauna (epigeic and endogeic, respectively), mesofauna, and microbial biomass were expected to be related to the plant functional traits and patch types. Site age and plant functional traits were more important explanatory factors for most of the chemical, microbial, and macrofaunal soil properties than the patch types. Patch type was the only plant-related factor capable of explaining the mesofauna community variations. Epigeic macrofauna were found to correlate more strongly with the plant functional traits than endogeic macrofauna. The abundance of most fauna correlated positively with an increase in plant cover and litter input and was significantly higher in vegetated patches and old sites.

1. Introduction

Plants modify the physical, chemical, and microbial properties of soil [1,2]. Moreover, they can create heterogeneity in space and time [3] which may well contribute to the habitat heterogeneity as plants are found at the bottom of the food web and can influence the development of soil, microhabitats, and other ecosystem components [4–6].

To explain the effects of vegetation on other ecosystem components, researchers have often used plant functional traits [7]. These functional traits such as plant size, plant form (e.g. grasses, forbs, legumes, and trees), and leaf properties can indicate the resource use, vegetation productivity, and the related leaf litter decomposition rate [8,9] in plants. Plant traits could explain part of the observed variation in communities of microorganisms [10] and arthropods [11] in and on the soil.

Most researchers have used plant functional traits obtained from databases, even though the predictive value may be greater for traits

directly observed at the particular field site [12,13]. Likewise, Frouz [14] pointed out that plant effects on other biota are driven, most likely, by quantitative parameters rather than a qualitative composition of the plant community. The explanatory power of plant functional traits, obtained as mentioned, is rarely compared with the in situ observed vegetation properties. Much less such assessment is done in a chronosequence to evaluate the potential differences that might occur in their explanatory power due to changes in plant species composition, demonstrated by soil chemical and biological variations.

Heterogeneity in post-mining spoil heaps is a typical characteristic of the revegetation during natural primary succession [15–17]. Hence, post-mining sites could provide chronosequence that offers an exceptional opportunity [4,17] to study such spatial and temporal heterogeneities in the vegetation. Here, we compared the use of plant functional traits with the use of vegetation height and density, expressed as patch type, to comparatively evaluate their explanatory power regarding the soil chemical, microbial, and mesofaunal and macrofaunal

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variations at four locations inside our post-mining site. The sites had different initiation dates thus creating a chronosequence. The dumped material of our site is considered to be homogeneous [16]. Our observation of plant properties in the field was quite broad, i.e. we resolved whether patches within sites were dominated by bare soil, grasses, or woody plants. We hypothesized that soil abiotic properties would be closely related to site age while soil faunal and microbial properties would be closely related to plant traits and to patch types. Regarding any variations explained through vegetation properties, we expected more of them to be related to the patch type as a more realistic evaluation of the in situ vegetation status than the database restricted plant traits. We also contemplated that the surface activity of macrofaunal communities, i.e. epigeic and endogeic, makes their variation differently related to the vegetation traits. Likewise, the difference in the immigration tactic, passive vs. active immigration, could cause a difference in the correlation of mesofauna and macrofauna, respectively, with the vegetation traits [18].

2. Materials and methods

2.1. Study sites

The study was carried out on large spoil heaps that had been deposited during open-cast coal mining in the western part of the Czech Republic (50°14'N, 12°40'E) in 2013. These post-mining sites were at an altitude of 550 m a.s.l. and had a mean annual precipitation of 650 mm and a mean annual temperature of 6.8 °C. The substrate consisted of tertiary clays [19]. Only unreclaimed (spontaneous succession) sites were used, and the sites had not been modified by human activity after spoil deposition. The four sites were 10, 18, 28 and 55 years old (age = time since heaping) and had extensions of approximately 0.3, 1, 2.7 and 1.1 ha, respectively. They were roughly 500 m from each other. Aerial photographs provided by the Czech Geodetic Institute were used to estimate the percentage of each site covered by each of the three patch types. In each site, the area covered by each patch type was manually plotted in ArcGIS and expressed as a percentage of the patch type area (Table S1). All of the main components of the plant community had been established at the beginning of the succession [4]. Herbs and grasses (*Tussilago farfara*, *Hieracium piloselloides*, and *Calamagrostis epigejos*) dominated at the 10-year-old sites. Although trees had been established early in succession, they did not dominate until later. Willow (*Salix caprea*) dominated at the 18-year-old site, and aspen (*Populus tremuloides*) and birch (*Betula pendula*) dominated at the 28 and 55-year-old sites.

At each successional stage, i.e. at each of the abovementioned sites, we tried to distinguish three types of patches: bare soil, grassy vegetation, and woody vegetation. Patches of woody and grassy plants were observed at all four sites, but patches of bare soil had been observed only at the two younger sites. Four plots were designated for each present patch type at each site, and subjected to macrofaunal, microbial, and chemical analysis. We determined plant species in a 1 m × 1 m area in each of the four designated plots for each patch type, if present, at each site. As a result of spoil heaping, the sites have undulating topographies consisting of waves of roughly 1 m in height with the top of the waves about 6 m apart. Our previous study [16] had shown that topography explains an important part of the variability. We eliminated the effect of the topography by using the same position on the waves, as only the slopes were sampled in the current study. The patches with different dominant vegetation are then the primary source of variation for most of the environmental variables in the given sites [16].

2.2. Sampling and processing

For analysis of soil chemical and microbial properties, five subsamples of soil, 0–5 cm below the litter layer, were taken from each plot

in June 2013; the subsamples were combined to form one 200 g composite sample per plot. The samples were transported to the laboratory, homogenized, and passed through a 2 mm screen. Part of each sample was immediately frozen (–40 °C) for microbial analysis and the rest were air-dried for chemical analysis. For analysis of soil macrofauna, we collected five subsamples of soil (area 125 cm², depth 5 cm including litter) from each plot; these were carefully combined to form one composite sample per plot and were kept at about 4 °C while they were transported to the laboratory. For analysis of soil mesofauna, we collected five subsamples of soil (area 19.6 cm², depth 5 cm including litter) from each plot; these were carefully combined to form one composite sample per plot and were kept at about 4 °C while they were transported to the laboratory.

A 1:5 soil:water extract was used for pH determination with a glass electrode and determination of electrical conductivity (EC) with a conductivity meter. Carbon (C) and nitrogen (N) content were measured using combustion gas chromatography (Elemental Analyser 1108, Carbo Erba, Italy). We extracted the available phosphorus (P) via Melich III solution. The P content in the extracts was then measured according to Watanabe & Olsen [20].

PLFA analysis was used to characterize the microbial community. Soil samples were extracted with a mixture of chloroform-methanol-phosphate buffer (1:2:0.8), according to Bligh & Dyer [21]. We separated the phospholipids using solid-phase extraction cartridges (LiChrolut Si 60, Merck) and subjected them to a mild alkaline methanolysis [22]. The free methyl esters of the PLFAs were then analyzed by gas chromatography–mass spectrometry (Varian 3400; ITS-40, Finnigan). We used the total content of all PLFA molecules as a measure of the total microbial biomass. The fungal biomass was quantified as the 18:2 ω 6,9 content, and the bacterial biomass was quantified as the sum of the following: G+ bacteria (sum of i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), G-bacteria (sum of 16:1 ω 7, 16:1 ω 5, 18:1 ω 7, cy17:0, and cy19:0), Actinobacteria (sum of 10 Me-16:0, 10 Me-17:0, 10 Me-18:0), and those markers which are not specific for any of the above mentioned groups (i.e. 17:0, 16:1 ω 9, 15:0, and 16:1 ω 7).

After extracting the mesofauna and macrofauna from soil samples with a Tullgren apparatus, we identified mesofauna, according to its external morphology. Springtails (Collembola) were grouped into three functional groups of euedaphics, hemiedaphics, and epedaphics, and mites into oribatids (Acari, Oribatida) and other mites (Acari). The macrofauna were identified to the family, orders or classes based on their external morphology and counted. Density of the macrofauna and mesofauna were expressed as number of individuals per m². Regarding the macrofauna, we recorded, and later on analyzed, the data about the larvae and adults separately, wherever applicable, as they can differ in ecology.

Percentage cover of individual plant species in the understory and in the tree and shrub layer were visually estimated in a 1 m × 1 m area in each plot. This step was taken in August 2013, when plants in the sites studied had achieved their maximal growth for the season. These were later on used to calculate the plant functional traits extracted from the database. Plant functional traits were calculated only for the understory plant communities, except for vegetation growth form that included trees and shrubs as well.

2.3. Data analysis

We determined the associations between dependent variables and patch type and site age with two-way ANOVAs using STATISTICA 10 [23]. When ANOVAs were significant ($P < 0.05$), the means were compared with the Tukey *post hoc* test.

We calculated the community-weighted means (CWM) for plant functional traits using the FD package [24,25] in R [26]. The computed values were then further analyzed with CANOCO. To assess the functional traits of the plant community, five plant traits related to essential plant functions were obtained from available databases. Seed mass and

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