



Dystric Cambisol properties at windthrow sites with secondary succession developed after 12 years under different conditions in Tatra National Park

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Abstract

Natural or anthropogenic disturbances in the forest ecosystem alter ecological conditions and lead to shifts in microbial diversity. We focused on the topsoil properties of specific sites in Tatra National Park (Slovakia) that were affected by windthrow, wildfire and wood extraction. We analyzed soil organic matter content (SOM), dry weight (DW), enzymatic activity including dehydrogenase activity (DHA), fluorescein diacetate hydrolysis (FDH) and phosphomonoesterase (PME). Bacterial community profiles were analyzed using the PCR-DGGE approach, and Ellenberg's indicator values (EIV) were used as eco-indices of studied sites. Dystric Cambisol was sampled at sites distinguished by management approaches established after windthrow in 2004 and wildfire in 2005. We focused on the windthrow site where wooden debris was extracted (EXT); the site left for self-recovery with no intervention (NEX); the site affected by wildfire (FIR), a former reference site damaged by the windstorm in 2014 followed by the extraction of wooden debris (REX), and a non-affected reference spruce stand (REF). The windthrow sites with different management (EXT, NEX) showed relative similarities based on enzymatic activity, bacterial community profiling (85%) and EIV comparison. The FIR site exhibited no similarities (0%) with EXT and NEX in the bacterial community structure. The SOM content correlated only with PME ($r^2 = 0.42$) and soil moisture ($r^2 = 0.64$). Based on RDA analysis, certain EIVs seem to be suitable indicators of selected soil properties. The lack of differences between NEX and EXT could be caused by the diminishing of post-disturbance effects on microbial communities. The effect of wildfire on microbial activity and bacterial community structure seems to be longer than different management approach.

Keywords TANAP · Enzymatic activity · EIV · SOM · windthrow · Wildfire

Introduction

The forest ecosystem is one of the dominant terrestrial ecological systems that evolved on the Earth and plays an essential role in maintaining biogeochemical cycles. Different kinds

of forests cover about 30.6% of the earth's surface area (FAO 2016) and the nature of biogeochemical cycles in these ecosystems is reflected in the quality of ecosystem services provided by forests. Soil represents an essential part of this scheme through the activity of organisms and specific chemical and physical properties (Baldrian 2017).

Natural or anthropogenic perturbations in forests cause shifts in soil properties affecting the biogeochemical cycles and thus also the quality of ecosystem services at the local, regional and even global levels. Twelve major perturbations were identified by (Práválie 2018) including phenological shifts, range shifts, die-off events, insect infestation, deforestation, fragmentation, air pollution, defaunation, fires, composition shifts, net primary productivity shifts, and biogeochemical shifts. The effects of perturbations on the forest ecosystem functions can be highly interconnected and lead to changes in biomass biodiversity or enhance shifts in carbon storage

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functions, which in many cases require decades or even centuries to be restored to their initial states (Certini 2005; Trumbore et al. 2015).

Interconnections among plants, soil properties, and enzymatic activity have been broadly studied, however, understanding the complexity of the soil environment and its response to perturbations continues to represent a scientific challenge (Burns et al. 2013; Luo et al. 2017). The extreme diversity of soil organisms contributes to a wide range of essential ecosystem services that are important for the sustainable functioning of natural and managed ecosystems. Soil organisms participate in carbon and nutrient cycles, soil structure modification and food web interactions that generate ecosystem services (Barrios 2007). Soil microorganisms provide decomposing organic matter, filtering water, stabilizing soil, generating and renewing soil fertility, releasing nutrients for plants, modifying the hydrological cycle, and controlling pests and pathogens of plants and animals (Wall and Knox 2014).

The functional diversity of soil microorganisms defines the categories and amounts of produced enzymes and thus affects also soil formation and transformation processes which play an essential role in the preservation of soil chemical and physical properties (Torsvik and Øvreås 2002). Perturbations in forest ecosystems may alter ecological conditions and result in shifts of microbial functional diversity (Prävšie 2018).

Changes in the microbial community structure are usually reflected in soil enzymatic activity. Several soil bioindicators can be used to identify alterations in the soil environment, however, the development of effective and widely applicable bioindicators continues to be a challenging task (Pulleman et al. 2012; Bommarco et al. 2013). The microbial production of enzymes is a communication bridge among microbial cells, the abiotic environment, and plants. The enzymes are used to retrieve and process complex organic molecules into compounds that can be transferred to the cell and serve as an energy source in energetic metabolism (Miralles et al. 2012; Grosso et al. 2014). Links among enzymatic activity, plants, and soil properties are mostly discussed in papers focused on agricultural land and crop production. Dehydrogenase and phosphatase activity can serve as bioindicators of changes in agroecosystems caused by soil amendments or agricultural practices (Moreno et al. 2001; Chu et al. 2007; Huang et al. 2009; Delgado-Baquerizo et al. 2016).

The aim of this paper was to analyze the interconnections among the enzymatic activity of soil microbiota, vegetation cover and soil properties in Dystric Cambisol. We focused on evaluating soil properties and analyzing soil bacterial communities in a mountain forest area 12 years after windthrow and wildfire and tried to trace the impact of applied management interventions (natural regeneration, logging). We were also interested in whether site conditions indicated by the plant community could serve as indicators of selected soil properties.

We hypothesized that

1. enzymatic activity will vary depending on the type of vegetation and forest management
2. enzymatic activity will reveal a strong correlation with the amounts of soil organic matter
3. forest management practices at the studied windthrow sites induced significant shifts in bacterial soil communities

Material and methods

Study area and sampling sites

Our study area was situated in the northern part of Slovakia in Tatra National Park (TANAP) (Fig. 1). Five sampling sites were selected according to the type of natural disturbance (windthrow, wildfire), management approach (dead wood extraction, self-recovery) and prevalent vegetation type (Table 1):

- | | |
|-----|---|
| EXT | windthrow site damaged in 2004 with subsequent salvage logging and removal of the fallen trees shortly after the disturbance, this site was reforested by <i>Picea abies</i> , <i>Pinus cembra</i> , <i>Larix decidua</i> , <i>Fagus sylvatica</i> , and <i>Acer pseudoplatanus</i> . |
| NEX | windthrow site damaged in 2004, no debris removal, left for a natural regeneration |
| FIR | windthrow site damaged in 2004 followed by the wildfire in 2005, the fallen trees were removed and the area was reforested with the same species as EXT. |
| REX | former reference site damaged by another, less severe windstorm in 2014 with the subsequent removal of the fallen trees. |
| REF | reference site established in 2014 represented by intact forest spruce stands (<i>Picea abies</i>). |

The elevation of the sampling sites ranges from 1045 to 1222 m a.s.l. All sites are located on slopes with a 5–10% gradient with south and southeast orientation. The soil type is identified as Dystric Cambisol. The soil texture is loamy sand with dominating sand 50.8–59.2% and silt particles 25.7–37.2% (Mičuda et al. 2006). The glade vegetation developed on the post-disturbance sites in the process of secondary succession, with vegetation of the alliance *Carici piluliferae-Epilobion angustifolii* followed by the vegetation of the *Sambuco-Salicion capreae* alliance. In addition to the native species of the spruce forest vegetation (e.g. *Avenella flexuosa*, *Calamagrostis villosa*, *Luzula luzuloides*, *Maianthemum bifolium*, *Melampyrum sylvaticum*, and *Vaccinium myrtillus*) other typical glade species are present

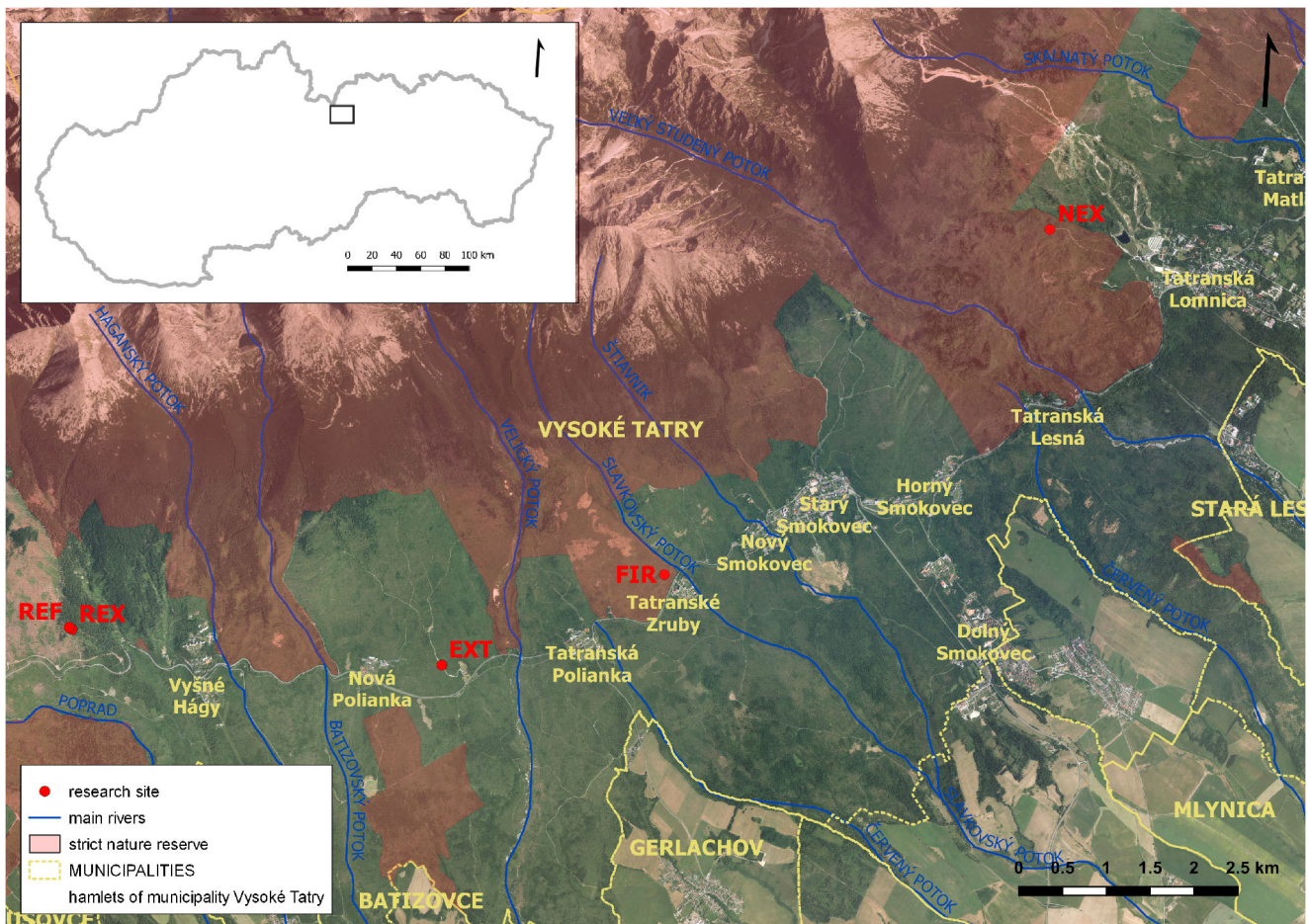


Fig. 1 Study area and the positions of sampling sites in the area of TANAP affected by windthrow and wildfire. EXT – extracted site; NEX – not extracted site; FIR – burned and extracted site; REX – previous reference site, extracted; REF – reference site

on the post-disturbance sites (e.g. *Calamagrostis villosa*, *C. arundinacea*, *Calluna vulgaris* (EXT and FIR sampling sites) and *Epilobium angustifolium*). More recently, several shrubs and light-requiring species as *Rubus idaeus*, *Salix*

caprea, and *Sorbus aucuparia*, as well as numerous populations of regenerating spruce (*Picea abies*), are developing. Secondary spruce forests are classified within the *Vaccinio myrtilli-Piceetum* association. The differences

Table 1 Main characteristics of sampling sites

Sampling site	Localization	Coordinates	Altitude (m a.s.l.)	Sampling point according to dominant vegetation cover	n
EXT	Danielov Dom	49°07'12.0"N 20°09'47.5"E	1045	<i>Calamagrostis villosa</i> (EXT C)	3
				<i>Rubus idaeus</i> (EXT R)	3
				<i>Picea abies</i> (EXT P)	3
NEX	Tatranská Lomnica	49°10'06.5"N 20°15'14.8"E	1074	<i>Rubus idaeus</i> (NEX R)	6
				<i>Picea abies</i> (NEX P)	3
FIR	Tatranské Zruby	49°07'50.4"N 20°11'49.4"E	1017	<i>Epilobium angustifolium</i> (FIR E)	3
				<i>Calamagrostis villosa</i> (FIR C)	3
				<i>Larix decidua</i> (FIR L)	3
REX	Vyšné Hágy	49°07'16.8"N 20°06'18.1"E	1217	<i>Vaccinium myrtillus</i> (REX V)	6
				<i>Avenella flexuosa</i> (REX A)	3
REF	Vyšné Hágy	49°07'17.5"N 20°06'16.4"E	1222	<i>Vaccinium myrtillus</i> (REF V)	9

EXT extracted site, NEX not extracted site, FIR burned and extracted site, REX previous reference site, extracted, REF reference site. n number of samples

between sampling sites are not significant. Above all, the vegetation of the post-disturbance sites can be characterized as acidophilous, less-nitrophilous with very low species diversity.

Soil sampling

The topsoil was sampled at 0–15 cm depth in July 2016 from the mineral horizon (A) excluding organic (O) horizon. In total, nine samples were collected from the area 15 × 15 m (NEX, EXT, FIR) or 20 × 20 m (REX, REF) at each site according to the dominant type of the vegetation cover based on phytocoenological relevés (Table 1). The fresh soil samples were sealed in plastic bags and kept at 5 °C in a portable container to ensure the minimal impact on enzyme activity for the temperate soils (Burns et al. 2013). The fresh soil samples were then sieved in a laboratory using 2 mm mesh and stored in a refrigerator at 4 °C.

Soil analyses and methodologies

We analyzed 45 individual soil samples for dry weight percentage (DW %), soil organic matter content (SOM) and enzymatic activity. The DW% was determined by drying soil samples at 105 °C overnight (Pansu and Gautheyrou 2007). The SOM content (%) was measured gravimetrically as a loss on ignition in samples dried at 550 °C during 4 h (Pansu and Gautheyrou 2007). The potential enzymes activities were determined by recommended standard assays and estimated colorimetrically. The hydrolytic capacity of the soil was estimated by fluorescein diacetate hydrolysis (FDH) in potassium phosphate buffer (pH = 7.6), according to the method originally described by Schnürer and Rosswall (1982) adapted by Shaw and Burns (2005) and optimized by Green et al. (2006). FDH was expressed as µg fluorescein/g dry soil/48 h. Dehydrogenase activity (DHA) was measured in Tris (hydroxymethyl) aminomethane (TRIS) buffer (pH = 7.0) according to the method originally described by Benefield et al. (1977) modified by Trevors (1984) and VonMersi and Schinner (1991) and expressed as µg INTF/g dry soil/48 h. The potential phosphatase (monoesterase) activity (PME) was determined according to Tabatabai and Bremner (1969) using a non-buffered system by distilled water instead, with Eivazi and Tabatabai (1977) and Shaw and Burns (2005) modifications. PME activity was expressed as µg p-nitrophenol/g dry soil/h. The soil reaction (pH) was measured by a glass electrode in soil suspension with deionized water (pH_{H2O}) and 0.1 M KCl (pH_{KCl}). The soil temperature (°C) was measured in situ by thermometer and soil moisture (%) in situ by portable device HH2 moisture meter DELTA-T DEVICES (Cambridge, England).

DNA extraction and PCR-DGGE analysis of bacterial soil communities

For DNA extraction from the soil samples, we used the Power-Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, USA). The extraction of total DNA was performed according to the manufacturer's recommendations. The extracted DNA was stored at –20 °C and used as a template in a polymerase chain reaction (PCR). Polymerase chain reaction (PCR) is a method widely used in molecular biology to make copies of a targeted gene section, in this case, 16S rRNA that is typical for bacteria. By polymerase chain reaction with denaturing gradient gel electrophoresis (PCR-DGGE), we can distinguish the differences in the biodiversity of bacterial soil communities among sampled sites.

PCR was done in two steps. In the first step, PCR was performed by using universal 16S rRNA gene primers (27F, 1492R) for a long gene fraction (Table 2). Each 20 µl reaction mixture contained 1 µl (10 ng) of DNA extracted from soil samples, 2.0 µl 10× AccuPrime Pfx Reaction mix (Invitrogen, USA), 1.25 U AccuPrime Pfx DNA polymerase (Invitrogen, USA) and 0.5 µM of each primer. For PCRs we used a thermal cycler (LabCycler, Goettingen, Germany) with the following cycling conditions: 2 min of denaturation at 95 °C, 35 cycles of 20 s at 95 °C, 30 s at 53 °C, 1 min 40 s at 68 °C, and a final cycle of extension at 68 °C for 10 min. PCR products were separated by electrophoresis in a 1% (w/v) agarose gel (Merck, Germany) and stained with GelRed Nucleic Acid Gel Stain (Biotium, USA). DNA bands, approximately 1500 bp in size were excised and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

In the second step, purified DNA bands were used in PCR with universal 16S rRNA gene primers (350F, 920R) with GC clamp on forwarding primer (Table 2). This set of primers generated DNA segments approximately 234 bp in size. PCR amplification was performed using the HotStartTag Plus DNA Polymerase (Hot-Star; Qiagen, Hilden, Germany). Each PCR reaction contained 2.5 U Tag Polymerase, 1× PCR Buffer, 1.5 mM MgCl₂, 10 pmol each primer, 400 nM of each dNTP and 10 ng of DNA template. For DNA quantifications NanoDrop 2000 (ThermoScientific, Wilmington, USA) was used. The volume of the reaction mixture was adjusted to 50 µl with ultrapure water (Millipore, Merck, Germany). Reactions were amplified in a thermal cycler (LabCycler, Goettingen, Germany) using the following program: 95 °C for 5 min, 35 cycles of 94 °C for 1 min, 73.5 °C for 1 min, 72 °C for 30 s, annealing temperature in the first step decreased by 0.5 °C every one cycle and 20 cycles of 94 °C for 1 min, 63.5 °C for 1 min, 72 °C for 1 min and 72 °C for 10 min last extension. Electrophoresis was performed on 1% agarose gel (Merck, Germany). The fingerprints (originating from the same gel) were compared

Table 2 Primer sets used in this study. Number in parenthesis indicate the GenBank accession number

Probes	Sequence	Description
27F 1492R	5' AGAGTTTGATCCTGGCTCAG 3' 5' ACGGCTACCTTGTTACGACTT 3'	16S rDNA universal primers, positions 8–27 and 704–685 and 1512–1492, resp. in the <i>E. coli</i> K12 [NC_000913] numbering system; (Lane 1991)
350F 920R	5' CGCCCGCCGCGCGGGCGG GCGGGGCGGGGGCACGGGG GGCTACGGGAGGCAGCAG 3' 5' ATTACCGCGGCTGCTGG 3'	16S rDNA universal primers, positions 341–357 and 534–518 in the <i>Escherichia coli</i> K12 [NC_000913] numbering system; (Muyzer et al. 1993)

by using BioNumerics Version 6.1 (Applied Math) with Pearson correlation similarity calculation and unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm.

DGGE was performed by using DCode System (BioRad, USA) as described in (Muyzer et al. 1993) with the following modifications. The PCR products of roughly equal amounts (°500 ng) were loaded onto a polyacrylamide gel 8% [wt/vol] acrylamide (Serva, Germany) in 0.5× Tris-acetate-EDTA (AppliChem, Germany) (TAE), with a 30–60% denaturant gradient and 40% [vol/vol] deionized formamide (Sigma-Aldrich, USA), and electrophoresis was carried out in 0.5× TAE buffer at 62 V for 18 h at 60 °C. After electrophoresis, the gel was stained at room temperature for 20 min with Ethidium bromide (Merck, Germany) and destained in distilled water for 20 min. The final gel was visualized by UV (Syngene, UK).

Phytocoenology of sampling sites

In June 2016, there were in total of 5 phytocoenological relevés from each site. The windthrow and the wildfire disturbed sites (EXT, NEX and FIR) were characterized by ongoing secondary succession and undersized stratum of tree layer with fine coverage. The area for phytocoenological relevés at NEX, EXT, and FIR represented 15 × 15 m. EXT site (15 × 15 m; 225 m²; E₁: *Avenella flexuosa* 3, *Calluna vulgaris* 3, *Calamagrostis arundinacea* 3, *Melampyrum sylvaticum* 2, *Luzula luzuloides* 2, *Epilobium angustifolium* 2, *Rubus idaeus* 2, *Maianthemum bifolium* 2, *Vaccinium myrtillus* 2, *Calamagrostis villosa* 2, *Vaccinium vitis-idaea* 1, *Larix decidua* 1, *Sorbus aucuparia* 1, *Picea abies* 1, *Sambucus racemosa* 1, *Galeopsis tetrahit* 1, *Veronica officinalis* +, *Salix caprea* +, *Senecio ovatus* +, *Hypericum maculatum* +, *Potentilla erecta* +, *Hieracium murorum* +, *Rumex acetosella* +, *Stellaria graminea* +, *Carex pallescens* +, *Hieracium lachenalii* r, *Silene flos-cuculi* r, E₂: *Picea abies* 2, *Sorbus aucuparia* 1, *Sambucus racemosa* 1, *Pinus sylvestris* +, *Larix decidua* +, E₃: 7%, E₂: 20%, E₁: 90%; 30.6.2016) was phytocoenologically characterized by E₃:

Picea abies 1, *Larix decidua* +, *Betula pendula* +, *Sorbus aucuparia* +, *Salix caprea* +.

The floristic composition of the NEX site (15 × 15 m; 225 m², E₃: 5%, E₂: 50%, E₁: 75%; 28.6.2016) was as follows, E₁: *Avenella flexuosa* 3, *Calamagrostis villosa* 3, *Luzula luzuloides* 2, *Rubus idaeus* 2, *Vaccinium myrtillus* 2, *Maianthemum bifolium* 1, *Epilobium angustifolium* 1, *Homogyne alpina* 1, *Calluna vulgaris* 1, *Melampyrum sylvaticum* +, *Galeopsis tetrahit* +, *Dryopteris filix-mas* +, *Dryopteris dilatata* +, *Salix caprea* +, *Sambucus racemosa* +, *Picea abies* +, *Sorbus aucuparia* +, *Dryopteris carthusiana* +, *Larix decidua* +, E₂: *Rubus idaeus* 3, *Picea abies* 2, *Larix decidua* 1, *Sorbus aucuparia* +, E₃: *Picea abies* 1, *Betula pendula* +, *Sorbus aucuparia* +, *Salix caprea* +.

The FIR site (15 × 15 m; 225 m², E₃: 7%, E₂: 9%, E₁: 90%; 30.6.2016) was characterized by E₁: *Avenella flexuosa* 3, *Luzula luzuloides* 2, *Epilobium angustifolium* 2, *Vaccinium myrtillus* 2, *Calluna vulgaris* 2, *Calamagrostis villosa* 2, *Calamagrostis arundinacea* 2, *Rubus idaeus* 1, *Maianthemum bifolium* 1, *Picea abies* +, *Senecio ovatus* +, *Hypericum maculatum* +, *Hieracium lachenalii* +, *Populus tremula* +, *Sorbus aucuparia* +, *Sambucus racemosa* +, *Veronica chamaedrys* +, *Taraxacum* sect. *Taraxacum* +, *Veronica officinalis* +, *Dryopteris carthusiana* r, *Campanula persicifolia* r. The wildfire affected site was characterized by pyrogenic succession (regeneration of vegetation after a wildfire), E₂: *Rubus idaeus* 1, *Picea abies* 1, *Larix decidua* 1, *Salix caprea* 1, *Populus tremula* +, E₃: *Larix decidua* 1, *Salix caprea* 1, *Betula pendula* +, *Pinus sylvestris* r.

The area for phytocoenological relevés at REX and REF sites represented 20 × 20 m. The REX (20 × 20 m; 400 m², E₃: 7%, E₂: 5%, E₁: 80%; 29.6.2016) and the REF (20 × 20 m; 400 m², E₃: 55%, E₁: 70%; 29.6.2016) sites were originally covered by forest spruce stands (*Picea abies*) and larch (*Larix decidua*). The floristic composition of the REX site was namely E₁: *Avenella flexuosa* 3, *Vaccinium myrtillus* 3, *Calamagrostis villosa* 2, *Luzula luzuloides* 2, *Epilobium angustifolium* 1, *Rubus idaeus* 1, *Maianthemum bifolium* 1, *Picea abies* 1, *Larix decidua* 1, *Homogyne alpina* 1, *Melampyrum sylvaticum* 1, *Vaccinium vitis-idaea* 1, *Trientalis europaea* 1, *Oxalis acetosella* +, *Sorbus aucuparia*

+, *Dryopteris dilatata* +, *Petasites albus* +, *Dryopteris carthusiana* r, *Salix caprea* r, *Gentiana asclepiadea* r. At the REF site, phytocoenological record revealed following floristic characterization E₃: *Picea abies* 3, *Larix decidua* 1, E₁: *Vaccinium myrtillus* 3, *Avenella flexuosa* 2, *Maianthemum bifolium* 2, *Homogyne alpina* 2, *Oxalis acetosella* 2, *Luzula luzuloides* 1, *Vaccinium vitis-idaea* 1, *Rubus idaeus* +, *Picea abies* +, *Sorbus aucuparia* +, *Larix decidua* +, *Veronica officinalis* +, *Trientalis europaea* +, *Dryopteris dilatata* +, *Epilobium angustifolium* r, *Dryopteris carthusiana* r, E₂: *Rubus idaeus* +, *Picea abies* 1, *Larix decidua* +, *Sorbus aucuparia* +, E₃: *Picea abies* 1, *Larix decidua* 1.

The cover of vascular plants was recorded using the seven-grade scale (Braun-Blanquet 1964). The relevés were stored in the database program Turboveg for Windows (Hennekens and Schaminée 2001). Sampling site conditions were estimated by bioindication using Ellenberg's indicator values (EIV) (Ellenberg et al. 1992) in Juice software (Tichý 2002). The syntaxonomical classification of the studied vegetation complies with Jarolímek et al. (2008). The nomenclature of vascular plants is according to Euro+Med (2006). Only species present in the herb layer were used for analysis.

Statistical analyses

Statistical analyses were performed using R software. Data are presented as mean ± standard deviation (SD). The comparisons among the different sites and vegetation units regarding the microbial and chemical soil properties were completed using the one-way analysis of variance (ANOVA) for normal distribution and the non-parametric Kruskal-Wallis test for the parameters that did not have a normal distribution. The Shapiro-Wilk test was applied to test the normality of data distribution and Levene's test was used to test the homogeneity of variances. As the intergroup comparison showed a significant difference, a post hoc Tukey's HSD test and Dwass-Steel-Critchlow-Fligner test was used to identify pairwise differences. Relationships between parameters were evaluated using Pearson's correlation analysis. Data normality was assessed by Shapiro-Wilk test; linearity and homoscedasticity were examined using a scatter plot. Outliers were detected by boxplot and visually (scatter plot), followed and supported by Rosner's extreme studentized deviate test for multiple outliers. Detected outliers were excluded from the dataset.

Principal component analysis (PCA) was used to interpret relationships between the microbial and chemical soil properties and to investigate relationships between sites and studied properties. The assumption of multivariate normality was assessed via Mardia's multivariate skewness and kurtosis test and the Henze-Zirkler test. Factor scores for each principal component were analyzed using ANOVA. To test hypotheses regarding the importance of environmental factors in explaining variation in soil data, Redundancy Analysis

(RDA) was used. RDA was used to assess the relationship between known environmental variables and variation in the multivariate soil data. The Monte Carlo permutation test was used to test the statistical significance of the relationship between environmental variables and variation in studied soil properties. Forward model selection based on the Akaike information criteria (AIC) was used to identify the set of the most relevant environmental variables. R package vegan was used for both, PCA and RDA (Oksanen et al. 2018). The data were standardized to zero mean and unit variance, and the analysis was done on the correlation matrix.

Results and discussion

Enzymatic activity

Since dehydrogenases are oxidative intracellular enzymes, DHA can be used as a suitable indicator of microbial-driven redox reactions in soil (Burns 1977). DHA intensity increased at studied sites in the following sequence: REF < REX < FIR < NEX < EXT. This indicates more redox active soil environment at sites with longer ongoing secondary succession (FIR, NEX, and EXT) compared to REF and most recently damaged REX (Fig. 2a). In regard to vegetation type, DHA intensity increased in the following order V < R < L < E < A < P < C (Fig. 3a). This can indicate a reduced redox capacity of soil under shrub species (*Rubus idaeus*, *Vaccinium myrtillus*). Oxidative processes play a crucial role in SOM degradation and transformations. These processes affect geobiochemical cycles maintaining nutrition availability for plants (Kieloaho et al. 2016); however, DHA at the studied sites did not correlate with the SOM amounts of any of the analyzed properties (Table 4). The lack of correlation suggested the presence of other factors affecting DHA (Wolińska and Stepniowska 2012). When comparing the DHA intensity at the studied sites, a pairwise test revealed a significant difference between DHA at NEX vs. REX and DHA at EXT vs. REX ($p < 0.05$) (Table 5). This difference indicates ongoing changes in the soil environment at the most recently disturbed REX site compared to other studied sites. However, these changes do not seem to be reflected in increased DHA (Zuber and Villamil 2016). No significant difference was measured among DHA at EXT, NEX, REF or FIR (Table 5) although a DHA data comparison with dominant vegetation species (Table 6) showed significant differences between DHA measured in soil sampled under *Rubus idaeus* vs. *Avenella flexuosa*, *Calamagrostis villosa* vs. *Vaccinium myrtillus*; *V. myrtillus* vs. *A. flexuosa* ($p < 0.05$), thus suggesting a strong effect induced by the rhizosphere of these species at microbial DHA (Ahmadi et al. 2018).

Overall soil enzyme activity can be indexed by the rate of fluorescein diacetate hydrolysis (FDH) since FDH is

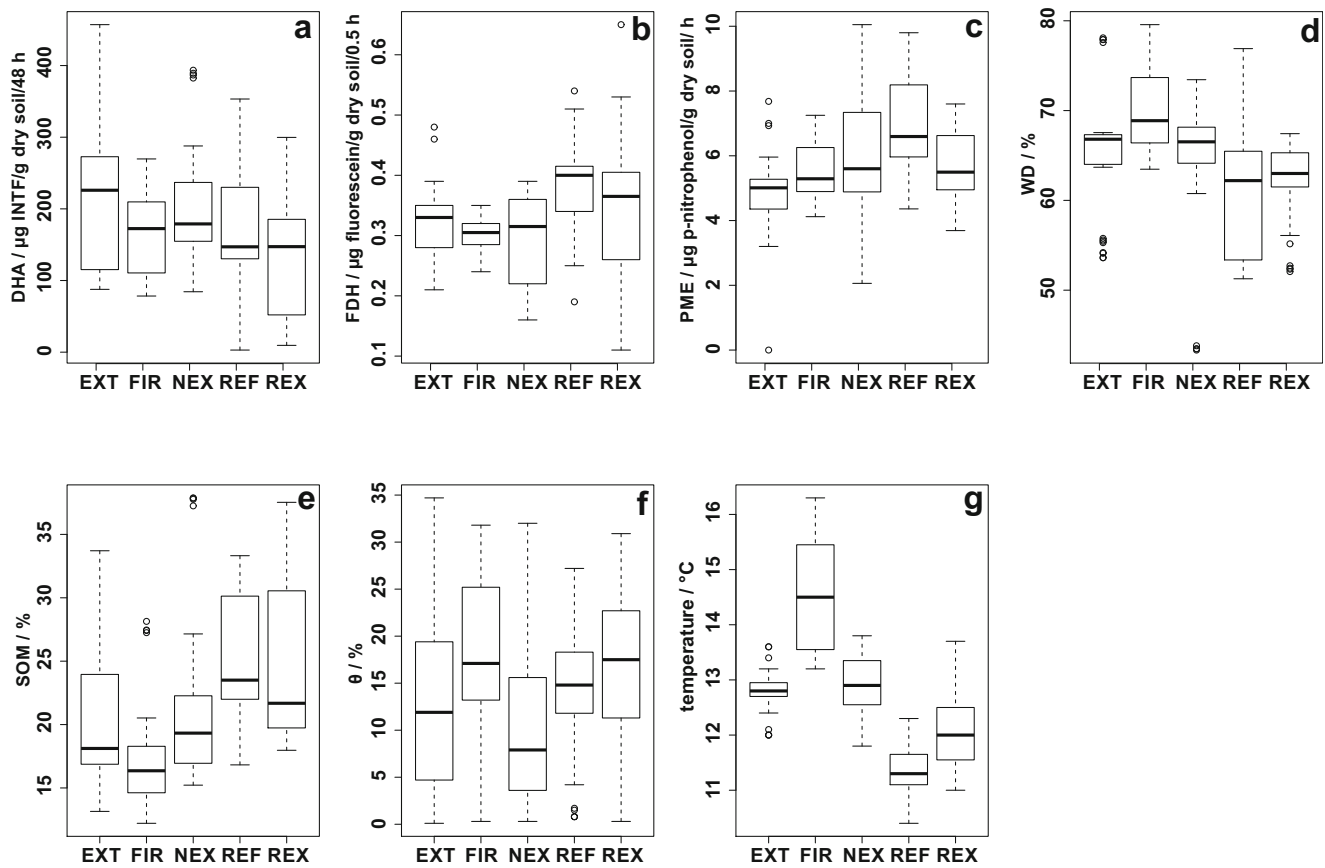


Fig. 2 Boxplots of study sites soil variables (a–g). SOM – soil organic matter, PME – phosphomonoesterase, FDH – fluorescein diacetate hydrolysis, DHA – dehydrogenase, DW – dry weight, θ – soil moisture,

EXT – extracted site; NEX – not extracted site; FIR – burned and extracted site; REX – previous reference site, extracted; REF – reference site

hydrolyzed by a variety of different enzymes (proteases, lipases, and esterases). An increasing trend in average FDH was observed as follows: FIR < NEX < EXT < REX < REF (Fig. 2b) and E < P < L < C < R < A < V (Fig. 3b). The windthrow and wildfire disturbed sites (FIR, NEX, EXT) showed lower values of FDH activity comparing to the REX and REF sites, thus indicating a decrease in overall enzyme activity (Schnürer and Rosswall 1982). When comparing FDH intensity at the studied sites, a pairwise test revealed significant differences between FDH at REF vs. NEX and EXT ($p < 0.001$) (Table 5) which can indicate the effects of natural disturbance or consequent forest management practices on soil hydrolytic capacity (Salazar et al. 2011). Surprisingly, the EXT site exhibited slightly higher FDH activity compared to the NEX site; however, this finding was not statistically significant. The minor differences among sites that indicated changes caused by forest management could eventually diminish in time due to ongoing secondary succession. The FDH values measured in topsoil under different vegetation cover showed the lowest activity at the FIR site (*Calamagrostis villosa*, *Epilobium angustifolium* and *Larix decidua*) (Fig. 3b, Table 3). The topsoil under *Vaccinium myrtillus* cover at the REF locality differed significantly compared to all of the windthrow and

wildfire disturbed localities (NEX, EXT, FIR, REX) (Table 5, Table 6). The FDH measured at sites where *Picea abies* dominated was significantly different from sites with dominant *Avenella flexuosa* and *Vaccinium myrtillus* ($p < 0.05$) (Table 6). Due to its complex function in the soil environment, FDH can be linked to other soil parameters (e.g. SOM) or vegetation cover (Oliveira et al. 2007; Venson et al. 2017). In our case, we observed a minor correlation between SOM and FDH ($r = 0.22$), that were not statistically significant (Table 4).

Phosphomonoesterase play an important role in the mineralization of organic P to inorganic form, which can be eventually utilized by plant roots and soil microbiota. We observed increasing PME activity in the following sequence: L < R < C < P < V < E < A (Fig. 3c). Higher enzyme activity could be recorded in the rhizosphere compared to bulk soil, which suggests the importance of this enzyme in plant nutrition (Karaca et al. 2010). We observed increasing average PME activity in the following order: EXT < FIR < NEX < REX < REF (Fig. 2c). Due to its strong linkage with vegetation, PME activity could be influenced by forest management strategies such as logging or harvesting wooden residues. Adamczyk et al. (2015) observed an increase in acid phosphatase activity linked to higher amounts of logging residue. When

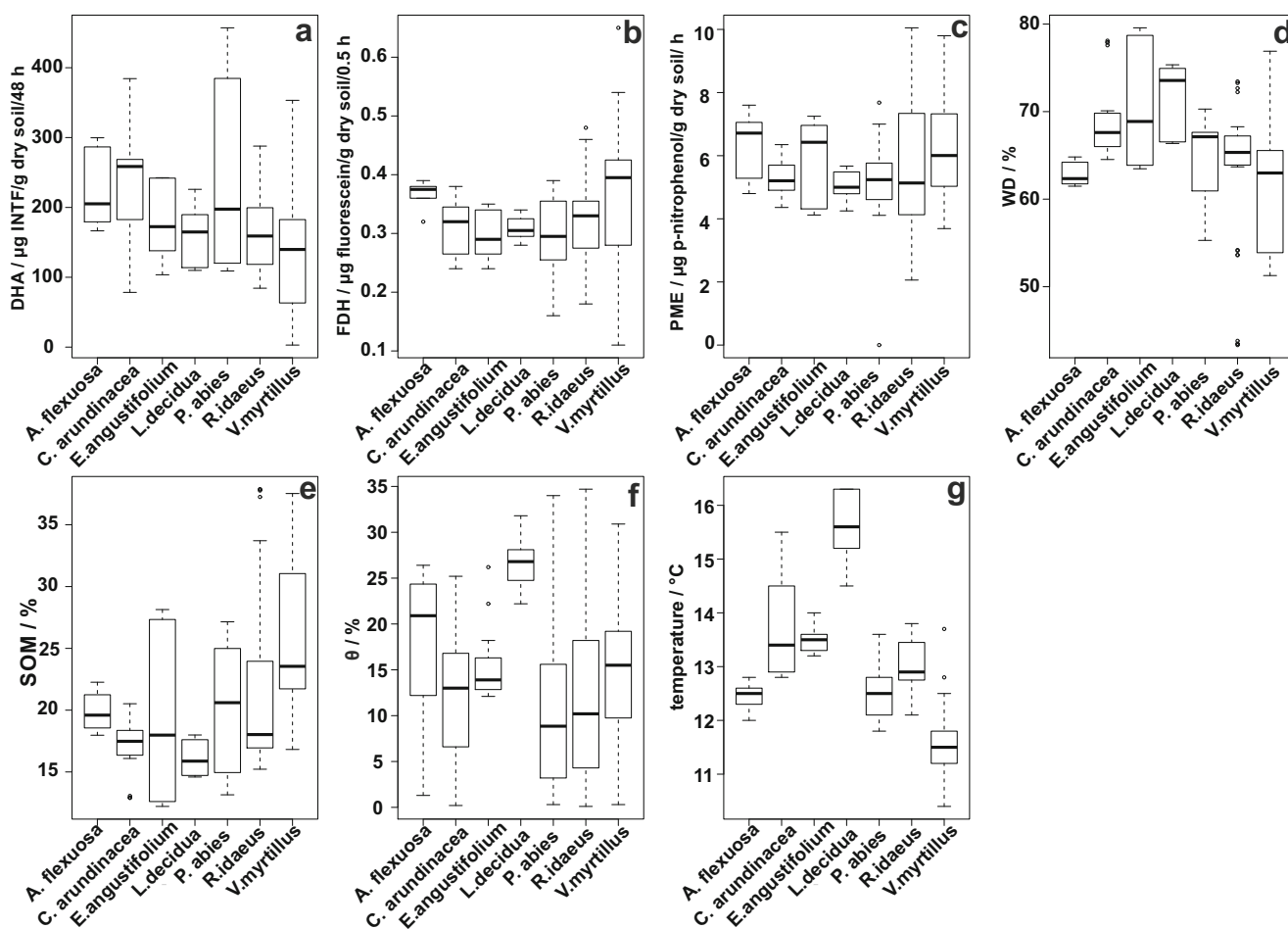


Fig. 3 Boxplots of different vegetation soil variables (a–g). SOM – soil organic matter, PME – phosphomonoesterase, FDH – fluorescein diacetate hydrolysis, DHA – dehydrogenase, DW – dry weight, θ – soil moisture

comparing the PME intensity at the studied sites, a pairwise test revealed a significant difference between PME at all extracted sites (EXT, REX, FIR) ($p < 0.001$) (Table 5). There

was a significant difference in PME activity in regard to vegetation cover (*Calamagrostis villosa* vs. *Avenella flexuosa*) (Table 6). The highest activity was observed at *Avenella*

Table 3 Data table of analyzed variables within different vegetation type

	DHA $n = 45$	FDH $n = 45$	PME $n = 45$	DW $n = 45$	SOM $n = 45$	Θ $n = 45$	T $n = 45$	pH _{H2O}	pH _{KCl}
EXT C	297.80 ± 18.08	0.32 ± 0.012	5.10 ± 0.015	70.24 ± 0.19	17.80 ± 0.19	10.63 ± 2.20	13.02 ± 0.08	3.98	3.36
EXT P	216.50 ± 43.55	0.30 ± 0.14	4.88 ± 0.55	63.36 ± 1.68	17.75 ± 1.58	13.47 ± 2.55	12.43 ± 0.17	4.13	3.93
EXT R	152.89 ± 17.17	0.32 ± 0.02	4.54 ± 0.26	61.77 ± 1.71	24.80 ± 1.94	14.15 ± 3.01	12.9 ± 0.06	3.89	3.06
NEX R	173.61 ± 12.06	0.30 ± 0.01	6.20 ± 0.5	63.41 ± 1.60	21.08 ± 1.60	11.45 ± 1.64	12.91 ± 0.12	3.68	3.24
NEX P	263.0 ± 27.63	0.27 ± 0.02	5.42 ± 0.12	66.07 ± 0.88	22.56 ± 0.88	9.78 ± 2.28	12.97 ± 0.14	4.21	3.31
FIR L	160.90 ± 11.21	0.29 ± 0.00	5.06 ± 0.14	72.65 ± 1.14	16.88 ± 0.38	15.48 ± 1.61	13.51 ± 0.26	4.56	3.45
FIR C	174.31 ± 21.95	0.30 ± 0.01	5.42 ± 0.16	67.35 ± 0.65	16.76 ± 0.90	13.06 ± 0.52	14.62 ± 0.22	4.79	3.62
FIR E	172.58 ± 21.58	0.31 ± 0.12	5.93 ± 0.37	70.96 ± 1.93	20.46 ± 1.89	13.06 ± 1.03	14.62 ± 0.08	4.63	3.35
REX A	220.79 ± 15.4	0.37 ± 0.00	6.37 ± 0.29	62.86 ± 0.37	19.85 ± 0.42	9.78 ± 2.06	12.97 ± 0.08	4.12	3.12
REX V	92.86 ± 12.73	0.34 ± 0.03	5.26 ± 0.18	61.51 ± 1.12	26.95 ± 1.30	11.45 ± 1.58	12.91 ± 0.15	3.93	2.87
REF	176.76 ± 16.02	0.39 ± 0.16	6.87 ± 0.18	60.90 ± 1.26	25.59 ± 0.80	14.806 ± 0.98	11.38 ± 0.10	3.85	3.81
S-W test	0.27	0.79	0.14	0.06	0.20	0.13	0.27	0.35	0.98
Skewness	0.51	0.13	0.47	-0.18	0.28	0.43	0.02	0.48	-0.02
Kurtosis	0.02	0.28	0.01	-0.21	-0.81	0.01	-0.17	-1.29	-0.96

SOM soil organic matter, PME phosphomonoesterase, FDH fluorescein diacetate hydrolysis, DHA dehydrogenase, DW dry weight, θ – soil moisture, T soil temperature, pH soil reaction, EXT extracted site, NEX not extracted site, FIR burned and extracted site, REX previous reference site, extracted, REF reference site. C – *Calamagrostis villosa*, P – *Picea abies*, R – *Rubus idaeus*, L – *Larix decidua*, E – *Epilobium angustifolium*, A – *Avenella flexuosa*, V – *Vaccinium myrtilillus*, S-W test – Shapiro-Wilk normality test, \pm SE

Table 4 Correlation matrix (Pearson’s RR) for the chemical quality of soil parameters

	DHA	DW	SOM	FDH	θ	T	PME
DW	-0.17						
SOM	-0.18	-0.70					
FDH	0.28	-0.46	0.22				
θ	0.08	-0.18	0.06	0.25			
T	-0.09	0.48	-0.40	-0.49	-0.14		
PME	0.17	-0.51	0.42	0.61	0.06	-0.42	
pH	0.05	0.38	-0.36	-0.22	0.04	0.69	-0.17

Significant Pearson’s R values ($p < 0.05$) are in boldface. $n = 44$. SOM soil organic matter, PME phosphomonoesterase, FDH fluorescein diacetate hydrolysis, DHA dehydrogenase, DW dry weight, θ – soil moisture, T soil temperature, pH - soil reaction

flexuosa and *Epilobium angustifolium* sites (Fig. 3c, Table 3). A good correlation was revealed between FDA and PME ($r = 0.615$) (Table 4) since PME is a hydrolytic enzyme (Shaw and Burns 2005).

Generally, connections exist between the enzymatic activity of soil and site conditions originating from management practices and differences of vegetation cover (Karaca et al. 2010; Moreno-Cornejo et al. 2015). The removal of wooden residue can affect soil processes, especially those involved in nutrients transformation. Wooden debris generally increases phosphatase activity, which is closely linked to vegetation and thus can enhance the decomposition of logging residue (Adamczyk et al. 2015). Natural disturbances and windthrow management practices can be stressful to soil organisms and lead to an alteration of soil conditions that trigger the response

Table 5 Adjusted p -values of pairwise comparisons of sites for significant soil parameters

	DHA	FDH	PME	DW	SOM	θ	t
K-W test	0.027	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FIR-EXT	0.454	0.077	0.199	0.014	0.110	0.023	<0.001
NEX-EXT	1.000	0.769	0.070	0.997	0.836	0.937	0.896
REF-EXT	0.901	0.001	<0.001	0.027	0.006	0.804	<0.001
REX-EXT	0.042	0.529	0.033	0.005	0.002	0.330	<0.001
NEX-FIR	0.720	0.991	0.822	0.020	0.005	<0.001	<0.001
REF-FIR	1.000	<0.001	0.001	<0.001	<0.001	0.141	<0.001
REX-FIR	0.357	0.051	0.897	<0.001	<0.001	0.793	<0.001
REF-NEX	0.755	<0.001	0.138	0.149	0.003	0.149	<0.001
REX-NEX	0.043	0.076	0.980	0.012	0.059	0.034	<0.001
REX-REF	0.601	0.243	0.010	0.872	0.712	0.604	0.003

Significant Pearson’s R values ($p < 0.05$) are in boldface. K-W test Kruskal-Wallis test, SOM soil organic matter, PME phosphomonoesterase, FDH fluorescein diacetate hydrolysis, DHA dehydrogenase, DW dry weight, θ – soil moisture, T soil temperature, EXT extracted site, NEX not extracted site, FIR burned and extracted site, REX previous reference site, REF reference site

Table 6 Adjusted p -values of pairwise comparisons of vegetation for significant soil parameters

	DHA	FDH	PME	DW	SOM	θ	t
K-W test	<0.001	<0.001	0.010	<0.001	<0.001	<0.001	<0.001
C-A	0.999	0.001	0.035	<0.001	0.009	0.180	0.001
E-A	0.757	0.002	0.952	0.021	0.866	0.929	0.006
L-A	0.070	0.001	0.082	0.001	0.001	0.005	0.006
P-A	1.000	0.023	0.142	0.675	0.998	0.370	0.999
R-A	0.046	0.012	0.859	0.207	0.986	0.337	0.015
V-A	0.023	0.920	0.995	1.000	0.002	0.587	0.001
E-C	0.191	0.980	0.675	0.998	1.000	0.817	1.000
L-C	0.102	0.995	0.979	0.653	0.344	<0.001	0.006
P-C	1.000	1.000	1.000	0.650	0.239	0.999	<0.001
R-C	0.055	0.997	1.000	0.032	0.274	1.000	0.068
V-C	0.003	0.044	0.066	<0.001	<0.001	0.612	<0.001
L-E	0.978	0.976	0.709	0.999	0.929	<0.001	0.006
P-E	0.962	1.000	0.815	0.324	0.998	0.439	0.003
R-E	0.991	0.892	0.999	0.314	0.975	0.553	0.081
V-E	0.777	0.130	1.000	0.009	0.019	1.000	<0.001
P-L	0.585	1.000	0.992	0.102	0.324	<0.001	0.001
R-L	1.000	0.846	1.000	0.003	0.012	<0.001	<0.001
V-L	0.972	0.315	0.099	0.001	<0.001	<0.001	<0.001
R-P	0.391	0.994	1.000	0.828	0.999	1.000	0.039
V-P	0.083	0.020	0.104	0.060	0.004	0.508	<0.001
V-R	0.750	0.045	0.537	0.335	0.032	0.607	<0.001

Significant Pearson’s R values ($p < 0.05$) are in boldface. K-W test – Kruskal-Wallis test. SOM soil organic matter, PME phosphomonoesterase, FDH fluorescein diacetate hydrolysis, DHA dehydrogenase, DW dry weight, θ – soil moisture, T soil temperature, pH soil reaction, EXT extracted site, NEX not extracted site, FIR burned and extracted site, REX previous reference site, REF reference site. A – *Avenella flexuosa*, C – *Calamagrostis villosa*, E – *Epilobium angustifolium*, L – *Larix decidua*, P – *Picea abies*, R – *Rubus idaeus*, V – *Vaccinium myrtillus*

of the soil microbial community. In our case, the REF site exhibited the highest FDA and PME activity, probably due to less stress caused by windthrow or wildfire (Gömöryová et al. 2014). The recently disturbed site (REX) was significantly different ($p = 0.01$) compared to REF in terms of PME activity; this was probably due to shifts in microclimatic conditions caused by the windstorm in 2014 (Gömöryová et al. 2017; Hanajík et al. 2017). The relatively low intensity of DHA at the REX site can be explained by changes in microbiocenosis leading to lower enzymatic activity as a result of the recent windstorm (Hanajík et al. 2017). We did not observe any significant differences in measured enzymatic activity between the EXT and NEX sites, probably because of the gradual diminishing of post-disturbance effects on microbial communities (Holden and Treseder 2013; Gömöryová et al. 2017). In regard to enzymatic activity, the burning effect at the FIR site seemed to vanish over time; however, we found significant differences in FDH and PME activity between the

FIR and REF sites ($p < 0.001$). The impact of fire on microbial activity depends on its severity, time and duration. Wildfire leads to the reduction of the surface organic horizon (O) and the enrichment of the mineral (A) horizon by mineral and organic nutrients (Certini 2005). Since post-disturbance changes can weaken over time, there is a positive correlation between time since disturbance and ecosystem recovery, which can be more than 15 years for boreal forests (Holden and Treseder 2013).

DW, SOM, soil temperature, and moisture

The relative water content in soil samples can be reflected in dry weight percentage. Generally, low DW% can indicate high moisture content. We observed that average oven-dried soil weight increased in the following order: REF < REX < NEX < EXT < FIR (Fig. 2d) and in the context of vegetation cover A < V < R < P < C < E < L (Fig. 3d). When comparing DW % among the studied sites with different disturbances, a pairwise test revealed significant differences ($p < 0.001$) (Table 5). The wildfire and extracted site (FIR) values were significantly different from all studied sites. The recently disturbed and extracted site (REX) differed from the EXT and NEX sites. We could not observe any significant differences between REX and REF, or between NEX and EXT. In terms of vegetation cover, the DW % measured at *Larix decidua* vegetation was significantly different from *Avenella flexuosa*, *Rubus idaeus* and *Vaccinium myrtillus* (Table 6).

The average SOM content values tended to increase in the following order: FIR < EXT < NEX < REX < REF (Fig. 2e) and in regard to the following vegetation type: L < C < R = E < A < P < V (Fig. 3e). We recorded significant differences between the burned (FIR) vs. the NEX, REX and REF sites. We could not observe any significant difference between sites with different management (EXT vs. NEX). Focusing on the reference site (REF) and recently disturbed (REX), there were no significant differences in SOM content (Table 5). The soil moisture (θ) of the studied sites increased in the following sequence: NEX < EXT < REF < FIR < REX (Fig. 2f) and for vegetation cover: P < R < C < E < V < A < L (Fig. 3f). According to the pairwise comparison test, the burned site (FIR) was significantly different from both disturbed sites (NEX vs. EXT) (Table 5). In terms of soil temperature, we recorded differences among all studied sites, but we observed no difference between the disturbed sites (NEX vs. EXT) (Table 5). Soil temperature increased at the studied sites in the following order REF < REX < EXT < NEX < FIR (Fig. 2g) and in regard to vegetation cover: V < P < A < R < C < E < L (Fig. 3g).

Catastrophic and severe windstorms completely reduced tree canopy, changed the microtopography at the disrupted areas and consequently altered soil moisture conditions. The implementation of different forms of forest management (e.g.

self-regeneration, logging) directly or indirectly influences soil water conditions (Edwards-Jonášová et al. 2010; Michalová et al. 2017). More than a decade after windthrow, we did not observe any significant difference in DW, SOM content, soil temperature or moisture between the extracted and self-regenerated sites. However, shortly after wind disturbance, Simkovic et al. (2009) observed differences in field water content between the EXT and NEX sites. The effect of burning can be obvious at the FIR site, where the values were significantly different from those of the other sites in several parameters such as SOM content, DW and soil temperature. The changes in soil properties induced by fire can be short, long term or even permanent. Burning removes organic matter but in the longer term, it increases organic carbon content in upper soil horizons (Certini 2005). Gömöryová et al. (2008) observed an increasing tendency of organic carbon content at the FIR site 4 years after wildfire disturbance; this trend was still evident but differences in C % are slowly diminishing. Furthermore, fire can alter vegetation composition and thus have an impact on the creation of vegetation patches. Since fire usually completely destroys the tree layer, in the following years the area is colonized by heliophilous grass species. Gaps in canopy improve light conditions and increase evapotranspiration and consequently soil water content (Schume et al. 2003; Šir et al. 2014).

Seasonality should be taken into account when measuring actual soil moisture and temperature measured in situ. Due to high soil heterogeneity, these parameters manifest high spatial and temporal variability. Daily and annual fluctuations in soil temperature influence soil processes and affect plant growth. Soil temperature depends on meteorological conditions, site topography, soil water content (DW) and texture, vegetation cover and canopy structure. The heat emission from the soil surface fluctuates during the day, strongly depends on actual meteorological conditions and is limited by the canopy of trees (Paul et al. 2004). The highest variability of soil moisture normally occurs in winter and spring and slowly depletes in summer (May–August) (Wu and Jansson 2013). Soil temperature can be characterized by a similar seasonal pattern, with peaks in the snow-free period (August–September) (Niinistö et al. 2011). The seasonal and daily patterns of precipitation, as well as weather conditions during sampling, can have an impact on actual soil temperature and moisture. In order to avoid misleading interpretations, we sampled soils in July when the conditions are considered to be more or less stable. In fact, soil parameters measured in situ exhibit high spatial heterogeneity which should be taken into account.

SOM formation depends on chemical, physical and biotic factors. Soil microorganisms mediate 85–90% of SOM decomposition and are widely dependent on substrate quantity and quality, temperature, moisture, pH soil type and depth (Siles et al. 2016). We observed relations between SOM and pH ($r = -0.36$) and soil temperature ($r = -0.40$). Soil

microbiota highly depends on organic macromolecules derived from SOM and facilitates humification and mineralization processes (Shaw and Burns 2005). In this case, we observed relations between SOM and PME ($r = 0.42$). Statistically, the topsoil sampled at *Larix decidua* was different from that of most of the other vegetation types; however, this can be caused by sampling design, because we sampled *Larix* soil only once. For both parameters measured in situ, we assumed the lowest values at the REF site due to intact canopy and shading, but this assumption was confirmed only for soil temperature. In general, soil enzymatic activity, SOM formation, and microbial community composition strongly depend on water availability (Wolińska and Stepniwska 2012). DW values and soil temperature were lower at the reference (REF) and former reference (REX) sites compared to the windthrow and wildfire disturbed sites (NEX, EXT, FIR) which can indicate the positive effect of reforestation and natural regeneration reflected in higher water content and temperature at sites with ongoing secondary succession.

Clustering of DGGE profiles

We compared the DGGE profiles 16S rDNA (total bacterial community) amplified from the soil. The dendrogram resulting from the analysis of soil samples showed two distinct clusters (Fig. 4). According to the dendrogram, the disturbed sites (EXT and NEX) together with the old reference site (REX) and reference site (REF) fell in cluster I (80% relative similarity). The wildfire site (FIR) formed a separate cluster (cluster II). In cluster I, EXT was up to 85% similar to NEX while REX was up to 94% similar to REF.

During the past years, attention focused mainly on evaluation and systematics of the soil fungi monitoring sites of TANAP (Šimonovičová et al. 2014; Hanajík et al. 2016) or the total microbial community structure (PLFA) (Hanajík et al. 2015). In this study, we focused more on the soil bacterial community. The microbial response to extraction and consequent wildfire (FIR) can be reflected in the formation of the separate cluster that represents a 0% similarity. Microorganisms are sensitive indicators of the functioning of

soil ecosystems and they react differently to a changing environment; their response can affect microbial biodiversity (Goulden et al. 2011; Právělie 2018). Heating produced during wildfires generally affects the availability of nutrients, enhances the decrease of microbial biomass and results in shifts of microbial community composition. A fire can have a selective effect on some groups of microorganisms, but in general, soil fungi are more reduced than bacteria (Certini 2005). However, following a fire, the percentage of soil bacteria can rapidly decrease by 33.3% (Holden and Treseder 2013). All of these facts could have an effect on the separation of a burned site compared to the reference and managed sites. The absence of wildfire together with the ongoing process of secondary succession may cause a relatively high degree of similarity of the total bacterial community (approximately 80%) among the managed sites (EXT, REX), the naturally recovered site (NEX) and the reference site (REF). The time that is necessary for a forest ecosystem to recover differs according to climate, ecosystem type, intensity and type of disturbance (Thom and Seidl 2016). The dendrogram revealed that differences between the managed (EXT) and unmanaged (NEX) sites are slowly diminishing. In the case of the new windthrow managed site (REX), the dendrogram showed a high bacterial community similarity to the reference site (REF), probably because it served as a previous reference site.

Phytocoenological and ecological characteristics

The composition of the vegetation species at the self-regenerating site (NEX) in regard to the successional stage can be characterized as a mixture of early and late successional species. In 2016, the pioneer *Rubus idaeus*, and in the grass layer, *Avenella flexuosa* and *Calamagrostis villosa* dominated. We also recorded seedlings of *Picea abies* and *Vaccinium myrtillus*. The extracted site (EXT) was characterized by late successional *Picea abies*, pioneer *Rubus idaeus* as well as grass species (*Avenella flexuosa*, *Calamagrostis villosa*). The logging of wooden residue after the windthrow probably had an effect of species composition and biodiversity. A similar pattern was observed by Michalová et al. (2017) who



Fig. 4 Graphical demonstration of bacterial community fingerprint as a dendrogram. Relative percentage similarity (a) is shown on the scale above the dendrogram (b). EXT – extracted site; NEX – not extracted

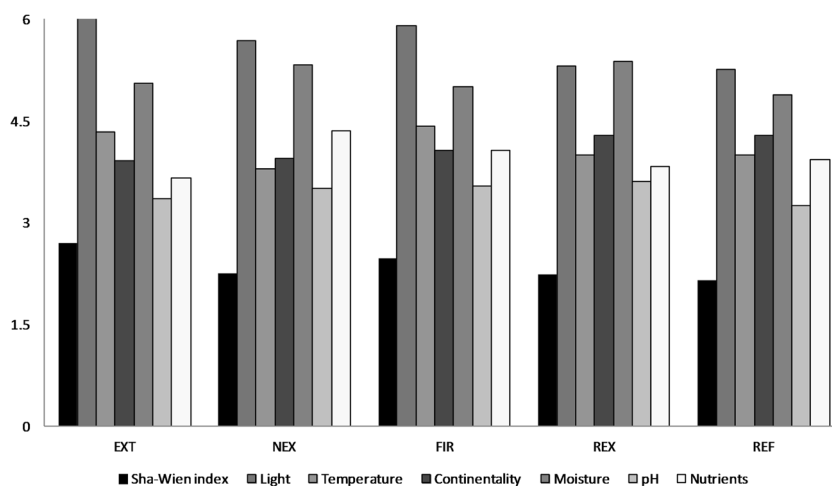
site; FIR – burned and extracted site; REX – previous reference site, extracted; REF – reference site

analyzed the impact of salvage-logging on spruce stands recovery in the Tatra Mts. A higher number of species together with increased diversity of grasses and pioneer species were detected on the salvage-logging site. In contrast, on the naturally regenerated site high shade tolerant species prevailed and had lower grass cover. The effect of extraction, as well as wildfire, could be reflected in the composition of the plant community and diversity at the burned site (FIR). The destruction of the canopy and enhanced sunlight can be associated with the presence of the heliophilous species (*Avenella flexuosa*, *Calluna vulgaris*, and *Larix decidua*). Moreover, we recorded an occurrence of nitrophilous and heliophilous *Epilobium angustifolium*. The wildfire in 2005 released soil nutrients and increased soil pH, which led to the prompt recolonization of the burned (FIR) site by *Avenella flexuosa* and *Calamagrostis villosa*. This could, in fact, have had a positive impact on its recovery (Certini 2005; Mičuda et al. 2006).

We used EIV to describe sampling sites according to the ecological demands of the species (Fig. 5). Generally, the Shannon-Wiener diversity index values were lower at the reference site (2.15) as opposed to the disturbed sites (2.25–2.69). Greater biodiversity values were recorded at the extracted site (EXT) in contrast to the self-regenerated site (NEX). The EIV values of temperature and light were greater at both extracted sites (EXT, FIR) as opposed to the non-extracted site (NEX). These sites were covered by light-demanding species such as *Avenella flexuosa*, *Calluna vulgaris*, and *Larix decidua*, probably because of gaps or the missing canopy. The reference site (REF) was characterized by greater continentality, probably due to the dominance of subcontinental *Picea abies* and *Larix decidua*. The vegetation at sites mainly covered by *Picea abies*, *Avenella flexuosa* and *Vaccinium myrtillus* (REF, REX) was strongly acidophilous in contrast to the FIR and EXT sites with semi-acidic *Epilobium angustifolium*. According to the EIV values, the recently (2014) disturbed site (REX) seems to have an association with both REF and EXT sites.

The vegetation of the Tatra Mts. is characterized not only by native forest communities but also by partly altered or non-native *Picea abies* monocultures 79 years old on average (Koren 2005). These old growth forest stands seemed to be more susceptible to wind disturbances. High severity disturbances are considered important drivers stimulating changes in the structure and complexity of forest stands (Čada et al. 2016). Wind disturbance leads to the fragmentation and opening of the forest canopy which generally has an impact on the functioning and biodiversity of the ecosystem. The windthrow followed by consequent logging of wooden residues alters early successional pathways that can consequently lead to the development of different forms of spatial complexity (Donato et al. 2012). The logging of wooden residue after windthrow probably has an effect on species compositions and biodiversity. Michalová et al. (2017) analyzed the impact of salvage-logging on spruce stands recovery in the Tatra Mts. A higher number of species and increased diversity of grasses and pioneer species were detected on the salvage-logging site. In contrast, high shade tolerant species which had a lower grass coverage prevailed on the naturally regenerated site. On the one hand, a naturally regenerated forest can be characterized by a higher number of young spruce, which is typically found in mosses and dead wood. Clearing operations led to a distribution of soil and vegetation and consequently to the expansion of grasses (Edwards-Jonášová et al. 2010). Retaining deadwood generally has a positive effect on humidity, shading and soil moisture (Marzano et al. 2013). On the other hand, when it comes to natural regeneration, this approach can provide a limited number of tree species. In this case, such low species diversity is typically seen in association with *Vaccinio myrtilli-Piceetum*; otherwise greater diversity richness at the NEX, EXT and FIR sites probably increased with the developing shrub layer (E₂) (Hanajík et al. 2016). Furthermore, the greater biodiversity values of the extracted sites (EXT, FIR) in contrast to the self-regenerated site (NEX) reflected the previous reforestation of this area (Fleischer et al.

Fig. 5 Ellenberg's indicator values (EIV) and Shannon-Wiener (Sha-Wien) diversity index of sampling sites. EXT – extracted site; NEX – not extracted site; FIR – burned and extracted site; REX – previous reference site, extracted; REF – reference site



2017). The wildfire on the extracted site (FIR) released soil nutrients and increased soil pH, resulting in prompt recolonization by *Avenella flexuosa* and *Calamagrostis villosa*. (Certini 2005; Hanajík et al. 2016).

The PCA analysis revealed a similarity in measured data among the REX, NEX, EXT and FIR sites (Fig. 6) and relationships between individual measured soil variables. The assumption of multivariate normality was confirmed (Mardia’s multivariate skewness p value = 0.16; Mardia’s kurtosis test p value = 0.42; Henze-Zirkler test p value = 0.26). The first two ordination axes explained 60.5% of the variance of the analyzed soil properties; axis-1 explains 42.6% and axis-2 17.9% of overall data variability. The first principal component (PC1) is most correlated with PME and the T. ANOVA of PC1 scores shows a significant influence of site management ($p < 0.001$). The pairwise comparison revealed significant differences between the REF and EXT, FIR, NEX sites ($p < 0.05$). PC2 was explained on the positive side by the SOM and on the negative side by the DHA. No different PC2 scores were found between different management.

As illustrated by PCA analysis (Fig. 6), the REF and FIR sites were significantly different from the others. PME and FDH had an impact on its graphical allocation and the FIR site lay aside due to soil temperature and DW. The highest PME and FDH activity was observed at the REF site, while the REX site had the lowest DHA. Furthermore, the FIR site had the lowest SOM content, but the highest DW and soil temperature. The NEX, REX and EXT sites were relatively

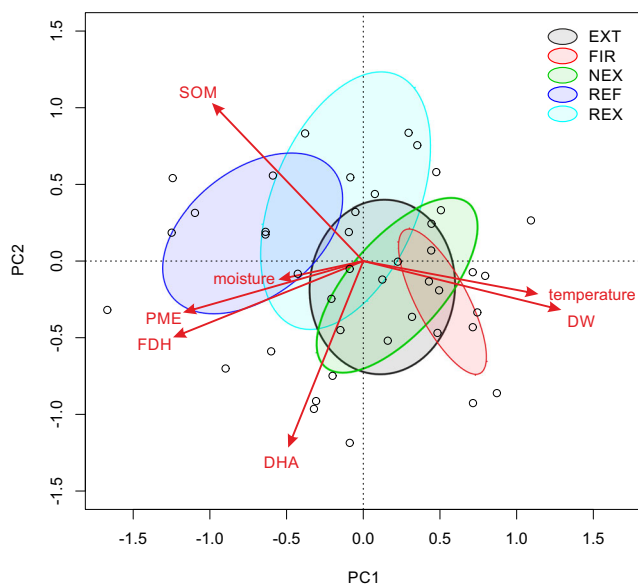


Fig. 6 PCA analysis showing differences of measured parameters among sampling sites. PC1 explains 46.6% and PC2 18.4% of overall data variability. EXT – extracted site; NEX – not extracted site; FIR – burned and extracted site; REX – previous reference site, extracted; REF – reference site. SOM – soil organic matter, PME – phosphomonoesterase, FDH – fluorescein diacetate hydrolysis, DHA – dehydrogenase, DW – dry weight

similar in measured parameters. The highest data variability and thus diversity of measured parameters were recorded at the NEX site, in comparison with the FIR site. For all data set, we found that DW negatively correlated with SOM ($r = -0.70$) and PME ($r = -0.51$). PME was positively correlated with FDH ($r = 0.61$). SOM correlated only with PME ($r = 0.42$). DHA had neither positive nor negative correlation with any of the analyzed parameters. The soil reaction (pH) did not correlate with any of the analyzed enzymes (Table 4).

The mutual relations between the analyzed data (DHA, FDH, PME, DW, SOM, θ , and t) and vegetation cover were explained by RDA analysis (Fig. 7). Ellenberg’s indicator values (EIV) were used to assess the spatial heterogeneity of the analyzed data. All EIV except nutrients and moisture were significant.

The Shannon-Wiener index of diversity and EIV of light positively correlated with DHA at the extracted (EXT) site. We can assume that windthrow together with consequent deadwood extraction and planting led to an expansion of heliophilous grasses that can accelerate the formation of ecosystems with high biodiversity and light demand (Michalová et al. 2017; Fleischer et al. 2017). The formation of permanent grass cover can increase enzymatic activity (Carpenter-Boggs et al. 2003) or change water demand (Šír et al. 2014). Continentality revealed a significant correlation with PME, FDH and SOM content at the reference (REF) site and the recently disturbed (REX) site. SOM structure widely depends on the litter chemical composition, which is a function of

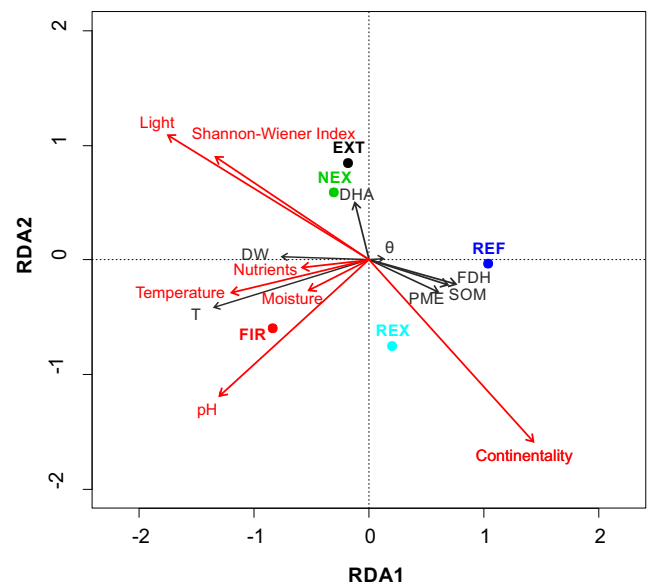


Fig. 7 The redundancy analysis (RDA) showing the relationship between the EIV, Shannon-Wiener (Sha-Wien) diversity index (red arrows) and the soil parameters (black arrows). RDA1 explains 21.0% and RDA2 3.7% of overall data variability. EXT – extracted site; NEX – not extracted site; FIR – burned and extracted site; REX – previous reference site, extracted; REF – reference site. SOM – soil organic matter, PME – phosphomonoesterase, FDH – fluorescein diacetate hydrolysis, DHA – dehydrogenase, DW – dry weight, θ – soil moisture, T – soil temperature

dominant plant species. The presence of *Picea abies* creates acidic soil conditions that slow litter decomposition and enhance the accumulation of undecomposed parts of SOM (Cotrufo et al. 2013; Špulák et al. 2016). The burned and extracted site (FIR) was characterized by species with a higher demand for nutrients, moisture, temperature, and pH. We can assume that the previous wildfire released soil nutrients and increased soil reaction (Certini 2005). The negative correlation between moisture EIV and moisture in situ is also interesting. This could be explained by the summertime measurement when soil moisture can decrease, and hydrophilous species could be still occurring.

Conclusion

In this paper, we focus on exploring the interconnections among soil enzymatic activity, vegetation cover and soil properties. Nevertheless, 12 years since windthrow and wildfire significantly influenced the area of the Tatra Mts. we were able to trace several differences arising from natural disturbance or applied forest management.

DHA intensity reached greater values at the windthrow and wildfire disturbed sites (EXT, NEX, FIR) compared to the reference site (REF). This can reflect increased degradation and transformation of SOM. However, we could not observe any significant difference in the intensity of DHA activity among sites with different management (NEX, EXT) or disturbance (EXT, FIR). On the contrary, the disturbed sites (NEX, EXT) exhibited decreased FDA and PME intensity compared to REF which can indicate the effect of windthrow as well as management practice on the hydrolytic capacity of the soil as well as the utilization of phosphorus by plants. We found a significant difference in FDH and PME activity between the FIR and REF sites, probably as a consequence of wildfire. In the context of overall enzymatic activity, we could not observe any significant differences between the windthrow affected sites with different management approaches (EXT and NEX); this is probably due to the gradual diminishing of post-disturbance effects on microbial communities. The wildfire seems to have had a long term impact on microbial activity than management (logging). The effect of vegetation cover on enzymatic activity was not as obvious as we expected, probably due to sampling design. However, we were able to find some significant differences. We observed significant differences between DHA measured in soil sampled under *Avenella flexuosa*, *Calamagrostis villosa*, *Rubus idaeus*, and *Vaccinium myrtillus*. The hydrolytic activity was significantly different at *Avenella flexuosa*, *Picea abies*, and *Vaccinium myrtillus*. We could not observe any significant differences in PME activity in soil sampled under different vegetation. In regard to SOM, we expected to find correlations between SOM and enzymatic activity. However, we could not

observe strong a correlation between DHA and SOM; we only recorded that SOM was slightly correlated with FDA and PME. In this case, we assume the presence of other factors affecting enzymatic activity (moisture, temperature, pH). The analysis of soil bacterial communities revealed differences between the wildfire (FIR) and windthrow affected sites (NEX, EXT). We were able to trace the impact of wildfire more than a decade after the disturbance, which seems to have had a greater effect on bacterial community structure than we expected. The relatively high similarity between sites with different management (EXT, NEX) suggests ongoing secondary succession with the gradual disappearance of post-disturbance effects. The differences between the recently disturbed (REX) and reference (REF) sites were small, but we can expect alterations as a consequence of opened canopy. In order to confirm these assumptions, it would be useful to have a detailed look at the bacterial community composition. The Shannon-Wiener index of diversity negatively correlated with SOM content and PME. For the purpose of this study, certain EIV seem to be suitable indicators of selected soil properties. Light conditions were negatively correlated with SOM, FDA, and PME. Temperature positively correlated only with DW. We found mutual relations between continentality and enzymatic activity on a large scale; however, moisture and temperature are considered to be more suitable predictors in small scales (e.g. soil environment).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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