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Microbial PLFA, Organic Carbon Fractions and Microbial Biomass in Soils under Different Windthrow Management in Biospheric Reservation of the Tatras

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Abstract

We analyzed phospholipid fatty acids (PLFA), carbon soluble fractions, holocelulose, klasonlignin and the microbial biomass carbon content in topsoils of the forest areas damaged by windthrow in the biospheric reservation of the Tatras in Slovakia. Soil ecosystem services and secondary succesion at the area were affected due to the instant wood debris removal. Topsoils were sampled nine years after the windthrow event at three plots selected according to geomorfological similarity and different forest windthrow treatments. EXT represented a site where all the wood biomass was removed, the NEX site was part of the area left for self-regeneration without stem and debris removal and as a control we sampled soils in a spruce stand (REF). Mol% of the lipids extracted from the soil samples were interpreted using the Principal Component Analysis (PCA) followed by the Analysis of Variance (ANOVA) and the Redundancy Analysis (RDA). The ANOVA analysis showed a significant treatment effect on the microbial communities at the studied plots ($P = 0.03$) and the PCA analysis showed similarity of the microbial community composition on the REF and NEX plot. The RDA analysis showed that the dry weight, loss of ignition and microbial characteristics were the most important factors in determining the PLFA microbial community composition ($\alpha = 0.05$).

Keywords: Analysis of variance, High Tatras, Microbial community, Phospholipid fatty acids, Windthrow

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Introduction

Windthrow is a universal phenomenon that occurs in most of the forest ecosystems and generally affects all ecosystem levels (Ulanova, 2000). Below- and above-ground communities of boreal forests participate in multiple interactions. All ecosystem components are in some way connected by processes that transform energy within the whole ecosystem. Windstorms generally form gaps in the tree canopy causing shifts in light intensity at the forest floor they also enhance nutrients availability, branches and stems of damaged trees, facilitate organic matter inputs to the ecosystem, and by forming pits and mounds uprooting, they alter the type of topography (Jonsson, 1993).

The logging of the damaged trees is generally considered to be one of the most significant factors that has a great influence on soil properties and causes changes in nutrient pools especially carbon and nitrogen cycles can adequately reflect the partial or total clearance of the trees and wood debris (Hazlett, 2007; Pereira et al., 2002; Thürig et al., 2005). Tree and debris removal cause shifts in underground waterflow, light intensity, temperature conditions or evaporation and thus have a strong influence on the soil properties (Rodney et al., 1994; Zerva et al., 2005) and induce alterations in composition of microbial communities (Leduc et al., 2007; Busse et al., 2006).

PLFAs, that are dominant components of the cell membrane, being promptly degraded after cell death, represent a suitable indicator of living organisms (White et al., 1979). PLFA methodology is nowadays widely used to expose microbial community structure and its variations, moreover according to the specific PLFAs relative quantity of some groups of soil organisms can be indicated (Zak et al., 1994, Zogg et al., 1997).

Soluble organic components of the litter are the first ones attacked by decomposing microorganisms and thus the first ones to disappeare. Substrates rich in soluble compounds consequently decompose more rapidly. The quality of decomposing wood debris is a significant aspect that controls the intensity of decomposition (Berg et al., 1982). An organic fraction that is easily soluble in water affects the early decay of different types of litter. (Gholz et al., 2000; Preston and Trofymow, 2000). Fatty acids, fatty alcohols, plant pigments, waxes, oils, resins or fats, that represent nonpolar organic extractables, can be removed and estimated using dichlormethane. Acetone, ethanol and hot water removes polar extractives soluble carbohydrates, ketones, pectins, tannins (Ryan et al., 1990; Wieder and Starr, 1998).

The main goal of this study was to elucidate plausible changes in the structure of the microbial community in topsoil at windthrow sites with different management treatments using organic fractions and microbial biomass as environmental variables. We performed experiments to assess the possible effects of these variables addressing questions of difference among studied plots comparing to the reference plot.

Materials and Methods

The study areas, described in detail by Hanajík and Fritze (2009), were located in the north part of the Slovak Republic in the TANAP forest zone damaged by windthrow. About 72.4% of damaged forest stand consisted of a 79-year-old Norway spruce (*Picea abies L.*) (Koreň, 2005). We sampled the topsoil at two sites with different treatments (EXT and NEX) and at the reference site (REF). The EXT sampling plot was part of a 8 038 ha area where all wood debris and stems were removed. The NEX sampling plot was part of a 699 ha non-intervention self regenerating area and the REF was part of an undamaged spruce forest located west of the windthrow. From each sampling plot (30 x 30m) 45 individual core samples of topsoil soil layer where combined into 3 composite samples. 15 individual core samples were included in each composite sample. After removal of visible plant material 9 composite samples were sieved using 2.0 mm mesh and were stored at 4 °C.

Moisture was measured in situ at each plot by a portable HH2 device. Dry weight (d.w.) was determined after drying soil samples at 105 °C during 24 hours. The content of soil organic matter was calculated as a loss of ignition at 550 °C upon 4 hours. To determine the dry weight (d.w.) of soil, samples were dried at 105 °C for 24 hrs. The soil organic matter content was measured as a loss on ignition from the dried samples at 550 °C for 4 hrs.

For the PLFA analysis we extracted lipids in 1.0 fresh soil (Bligh and Dyer 1959, White et al. 1979) applying chloroform-methanol-water buffer system (1:2:0.8) Using silicic acid we isolated neutral lipids, glycolipids and polar lypids according to King et al.(1977) and Kates (1986) Isolated phospholipids from lipid fractions were transesterified into fatty acid methyl ester (FAMEs) and detected by gas chromatograph with a flame ionization detector using a 50 m HP-5 phenylmethyl silicone capillary column. The carrier gas was Helium. The phospholipids were isolated from the lipid fraction and transesterified into fatty acid methyl esters (FAMEs) which were detected by gas chromatography (flame ionization detector) using a 50 m HP-5 (phenylmethyl silicone) capillary column. Helium was used as a carrier gas. The initial oven temperature increased from 50 ºC to 160 ºC at the rate of 30 ºC min^{-1} and was raised at a rate of 2 ºC min^{-1} up to a final rate of 270 ºC and was retained at this temperature for 5 minutes. PLFAs were identified according to the retention time index calculated as area % of summed peak area (Mol %) relative to the retention times of the 13:0 and 19:0 internal standards.

Organic nonpolar and polar fractions were analyzed in the topsoil according to the method developed for peat samples (Karsisto et al., 2002; Karsisto et al., 2003) Mass loss of nonpolar extractives was determined by dichlormethane extraction. Mass loss of polar extractives was determined by acetone. Ethanol and water extractions, klasonlignin and holocelulose were determined from extractive free residues and the acid soluble lignin was analysed from klason-lignin filtrate using Shimadzu 2401PC spectrophotometer.

Microbial biomass C was determined by chloroform fumigation extraction (CFE) according to Vance et al. (1987) using a membrane air pump

N810.3FT.18 with an automatic vacuum controller. For the CFE method, four subsamples of 10 g of all topsoils were fumigated with ethanol-free chloroform in a sealed desiccator in the dark for 24 h at 180 mbar and the four non-fumigated subsamples of 10 g of each topsoil were directly extracted with freshly prepared 0.5 M K_2SO_4. After fumigation desiccator was evacuated six times to remove the chloroform from the soils followed by the extraction of the samples.

The obtained data were assembled and edited in Microsoft Excel. Graphics and statistics were computed by R software, ver. 3.1.3, redundancy analysis (RDA) and principal component analysis (PCA) were computed and visualized by CANOCO for Windows, version 4.5. For the purpose of this study we used PLFAs as 'species' in PCA and RDA. The ordination emphasized interspecies correlations, standard deviation was used to divide species scores in order to diminish the influence of extreme values and the ordination centered by species and samples was favored. The studied sites (EXT, NEX and REF) are displayed as points in the ordination space (a distance diagram). 'Species' represented by PLFA response variables together with environmental variables were dysplayed as arrows that are projected from the origin (ter Braak and Prentice, 1988). We used ANOVA to analogize the means of all variables and identify significant differences among the sites. Tukey's HSD test was used to determine signification of post hoc pairwise comparisons. For the identification of correlations among variables, the linear Pearson's r coefficient was used and arcsine square root transformations were applied to variables that represented proportions of other variables. A level of significance $\alpha = 0.05$ was selected for all tests.

Results and Discussion

We analyzed amounts of 43 PLFAs (Table 1). PLFAs i15:0, a15:0, i16:0, 16:1ω9, 16:17t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0 are predominantly bacterial origin (Frostegård et Bååth, 1996) and 18:2w6 represented indicator of microscopic fungi (Frostegård et al., 2010). The ANOVA analysis of the Principal Component 1 (Axis 1) scores, showed significant treatment effects ($P = 0.03$) on the microbial communities at studied plots and the PCA analysis showed similarity of the microbial community composition on the REF and NEX plot. Samples at the EXT site were well separated from the REF and NEX site along axis 1 explaining 38.1% of the variation in composition of PLFAs that corresponds to a division due to the treatment site management (influenced largely by 18:1ω7, 18:1ω9, 18:2ω6 and 16:0) (Figure 1).

Table 1. *Amount of PLFAs Expressed as mol % in Topsoil at Study Localities. Expressed as means (n=3) ± SE.*

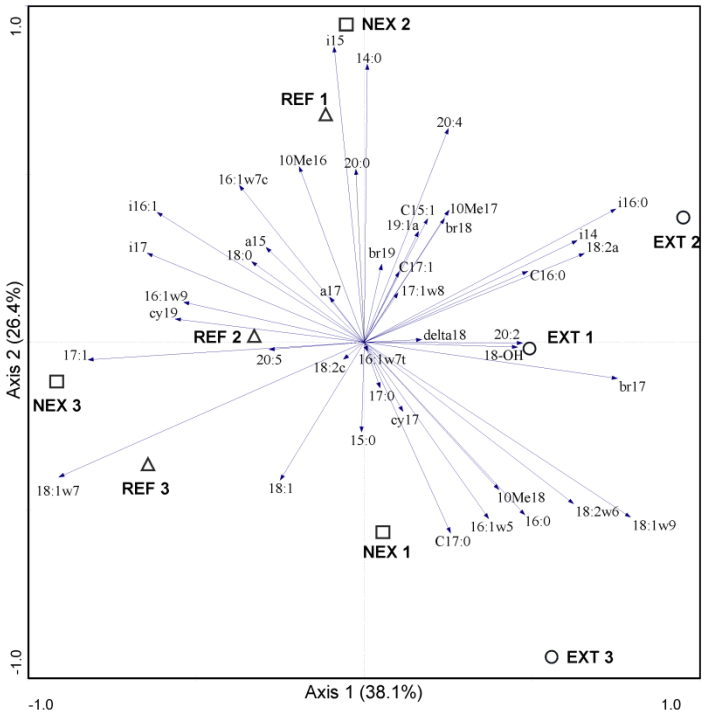
	i14:0	**14:0**	**i15:0**	**a15:0**	**C15:1**	**15:0**	**i16:1**	**C16:0**	**i16:0**	**16:1w9**	**16:1w7c**
EXT	0.43 ± 0.01	1.46 ± 0.05	9.75 ± 0.35	3.33 ± 0.08	0.45 ± 0.08	0.89 ± 0.02	1.08 ± 0.01	0.75 ± 0.04	5.8 ± 0.08	0.49 ± 0.04	7.58 ± 0.19
NEX	0.4 ± 0.02	1.53 ± 0.10	10. ± 0.42	3.48 ± 0.11	0.35 ± 0.03	0.83 ± 0.02	1.28 ± 0.04	0.68 ± 0.04	5.63 ± 0.18	0.46 ± 0.07	8.09 ± 0.17
REF	0.37 ± 0.02	1.42 ± 0.04	10.29 ± 0.46	3.3 ± 0.06	0.42 ± 0.02	0.95 ± 0.01	1.15 ± 0.03	0.61 ± 0.01	5.41 ± 0.13	0.65 ± 0.02	7.69 ± 0.06

	16:1w7t	**16:1w5**	**16:0**	**br17:0**	**17:1**	**10Me16:0**	**C17:0**	**i17:0**	**a17:0**	**17:1w8**	**cy17:0**
EXT	1.15 ± 0.01	2.95 ± 0.05	12.36 ± 0.18	0.27 ± 0.01	0.2 ± 0.01	7.04 ± 0.14	1.34 ± 0.42	1.38 ± 0.02	1.5 ± 0.03	0.42 ± 0.03	1.74 ± 0.03
NEX	1.01 ± 0.04	2.88 ± 0.09	11.4 ± 0.19	0.25 ± 0.01	0.28 ± 0.04	7.76 ± 0.34	1.49 ± 0.44	1.55 ± 0.07	1.5 ± 0.03	0.41 ± 0.04	1.43 ± 0.02
REF	1.19 ± 0.02	2.78 ± 0.07	11.57 ± 0.17	0.24 ± 0.00	0.24 ± 0.03	7.38 ± 0.34	1.08 ± 0.08	1.41 ± 0.03	1.61 ± 0.04	0.46 ± 0.03	1.81 ± 0.05

	C17:1	**17:0**	**br18:0**	**10Me17:0**	**18:2a**	**18:2w6**	**18:1w9**	**18:1w7**	**18:1**	**18:0**	**19:1a**
EXT	0.28 ± 0.01	0.45 ± 0.00	1.16 ± 0.05	1.39 ± 0.01	0.48 ± 0.07	2.95 ± 0.18	7.3 ± 0.15	9.9 ± 0.51	1.14 ± 0.02	2.04 ± 0.08	0.48 ± 0.03
NEX	0.22 ± 0.01	0.42 ± 0.01	1.2 ± 0.03	1.41 ± 0.07	0.29 ± 0.07	2.66 ± 0.55	6.25 ± 0.18	11.21 ± 0.59	1.2 ± 0.02	2.14 ± 0.05	0.42 ± 0.03
REF	0.32 ± 0.03	0.46 ± 0.01	1.04 ± 0.01	1.4 ± 0.01	0.41 ± 0.03	2.46 ± 0.02	6.27 ± 0.03	11.4 ± 0.44	1.15 ± 0.08	2.04 ± 0.07	0.46 ± 0.01

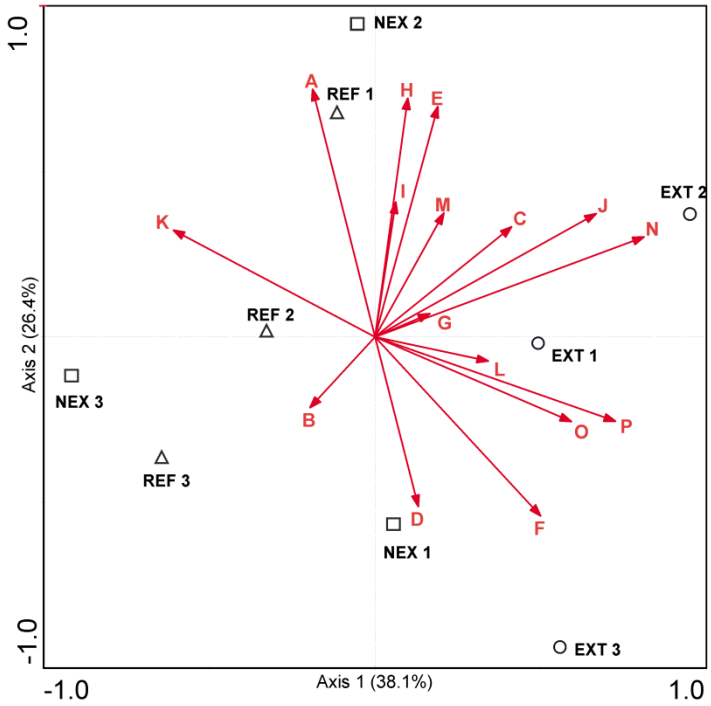
	18-OH	**10Me18:0**	**18:2c**	**br19:0**	**delta18:0**	**cy19:0**	**20:5**	**20:4**	**20:2**	**20:0**
EXT	0.11 ± 0.05	1.39 ± 0.03	0.08 ± 0.00	0.09 ± 0.00	1.5 ± 0.21	5.69 ± 0.21	0.66 ± 0.06	0.21 ± 0.03	0.23 ± 0.04	0.13 ± 0.01
NEX	0.02 ± 0.02	1.31 ± 0.1	0.35 ± 0.20	0.07 ± 0.01	1.05 ± 0.05	5.97 ± 0.32	0.57 ± 0.05	0.24 ± 0.04	0.16 ± 0.01	0.15 ± 0.04
REF	0.1 ± 0.02	1.37 ± 0.05	0.2 ± 0.12	0.1 ± 0.01	1.53 ± 0.13	5.77 ± 0.09	0.77 ± 0.07	0.17 ± 0.03	0.15 ± 0.02	0.41 ± 0.33

Figure 1. *PCA of the Obtained PLFAs Data Set for the 8 Soil Samples, Using 43 PLFAs as Species*



PLFAs data obtained by Hanajík and Fritze (2009) who analyzed topsoils at identical EXT, NEX and REF plots four years after windthrow showed significant treatment effect on microbial community ($P \leq 0.0016$). Tukeys test showed in 2009, the separation of microbial community group at REF from EXT and NEX treatments. Tukeys test in 2013 showed treatment EXT separeted from REF ($P = 0.034$) and NEX ($P = 0.043$) microbial community group. Tukeys test did not show the separation of the community at REF and NEX ($P = 0.98$). Accordingly to this data we can assume that forest management approach at plot NEX caused shifts in ecological conditions and soil characteristics resulting in faster restoration of soil characteristics on NEX comparing to EXT and consequently led at NEX to development of microbial community structure more similar to the reference plot. The direction of the maximum variation in the PLFAs in the ordination diagrams was visualized by arrows that expressed the magnitude of the shifts proportionaly to the arrows length. The most important PLFAs significant in explaining the sites dissimilarities were positioned nearby the plots edge while PLFAs of lower significance were adjacent to the center. The conjoint direction of the PLFAs arrows with arrows representing environmental characteristics suggested the correlation among variables. Confidence in correlation was proportional to the length of the arrows (ter Braak, 1994). Diagram showing the relationships among the PLFAs, environmental gradients and the samples was created by linear canonical community ordination method RDA (Figure 2).

Figure 2. *The Redundancy Analysis (RDA) of the PLFAs Data Set for the 8 Samples Using 43 PLFA as Species and 19 Environmental Variables*



(A) weight loss percentage (wl%) of dichlormethan extractables, (B) wl% of aceton extractables, (C) wl% of ethanol extractables, (D) wl% of water extractables, (E) % of klasonlignin content, (F) dry weight percentage (DW%), (G) % of acid soluble lignin content, (H) % of holocelulose content, (I) total wl% of extractables, (J) Organic matter content, (K) moisture (vol%), (L) carbon content in microbial biomass (fumigation) (Cmic-fumig), (M) total PLFA amount in nmol/gdw, (N) bacterial PLFA in nmol/gdw, (O) fumigal PLFA in nmol/gdw, (P) microbial biomass nmol/gdw.

Obtained PLFA data in this study did not correspond to the unimodal distribution, thus RDA linear model was used instead of the similar unimodal canonical correspondence analysis (CCA). The ordination axes which are constrained to represent linear combination in RDA enabled the direct comparison of the interrelations among the environmental variables and response variables. The longer arrows representing the environmental gradients endorsed more confidence in the environmental gradient certainty in the inferred correlations, approximately indicate a stronger affect of the variable on the overall variations of the species (ter Braak and Smilauer, 2002). The directions of the arrows represented the directions in which the site scores would have shifted if there was an increase of environmental variables. RDA analysis identified those specific environmental variables significantly associated with the PLFA variables, as tested by Monte Carlo permutation showing most significant environmental variables including N (P = 0.016), P (P = 0.032), F (P = 0.034), J (P = 0.034), and O (P = 0.042) (Figure 2). Variables tested as significant (N,R,P,J,M,F see figure 2 text) were positively correlated with Axis 1 and were higher at EXT site comparing to REF and NEX site.

Conclusions

The PLFA data in this study did not conform to an unimodal distribution. The ANOVA analysis of the scores of Principal Component 1 (Axis 1), did show a significant treatment effect (P = 0.03) on the microbial communities at studied plots and PCA analysis showed similarity of the microbial community composition on the REF and NEX plot. Samples at the EXT site were well separated from REF and NEX site along axis 1 explaining 38.1% of the variation in PLFA composition, corresponds to a division between treatment site management (influenced largely by 18:1ω7, 18:1ω9, 18:2ω6 and 16:0). Using the RDA analysis we identified most significant environmental variables including bacterial PLFA in nmol/gdw (P = 0.016), microbial biomass in nmol/gdw (P = 0.032), dry mass percentage (P = 0.034), organic matter percentage (P = 0.0034) and fumigal PLFA in nmol/gdw (P = 0.042). Variables tested as significant were positively correlated with Axis 1 and were higher at the EXT site comparing to REF and NEX site. We assume differences are slowly disapearing among study sites regarding the ANOVA analysis, but still some differences in soil characteristics can be identified. Wood debris removal approach at site EXT most likely caused more shifts in primary ecological conditions at this plot and led to a different evolution of soil properties comparing to NEX, where the non-removal approach resulted in primary conditions more alike conditions at REF and thus resulting in a higher similarity between the REF and NEX site.

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