Soil microbial community responses to windthrow disturbance in Tatra National Park (Slovakia) during the period 2006 – 2013

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Abstract

Soil microbial communities were studied in the Tatra National Park, which was affected by a windthrow in 2004 and by fire in 2005. The objective of the study was to compare the response of soil microorganisms to different management regimes on disturbed areas and to evaluate the microbial community changes during the period 2006–2013. Soil samples were taken from the A–horizon along 90 m transects on 4 plots (reference intact plot, plot with extracted wood, burnt plot, plot with fallen trees left in situ). Basal and substrate-induced respiration, microbial biomass carbon (C), nitrogen (N) mineralisation, catalase activity, and richness and diversity of microbial functional groups were determined in soil samples using the BIOLOG EcoPlates. Generally, the highest microbial activity and biomass C were revealed at the reference and fire plots. No distinct differences in microbial attributes were found between the extracted and non-extracted plots. At all windthrow plots, substrate-induced respiration, microbial biomass C and N–mineralisation showed a significant increasing linear trend with time what indicates a gradual recovery of microbial community at plots after windthrow.

Keywords: forest soil; microbial biomass; microbial activity; windthrow

1. Introduction

During the last decade, changes in climate have had impacts on natural and human systems across all continents and oceans (ICPP 2013). Climate changes are usually associated with increasing concentration of greenhouse gases which causes increase of air temperature and changes in precipitation; however, they can be accompanied also with other important changes, e.g. occurrence of extreme weather events such long lasting droughts, flash floods, thunderstorms, wind storms, heat waves, but also in expansion of pests, weeds, pathogens, dangerous insects, etc. (Lindner et al. 2010; ICPP 2013).

Windthrows belong to the most important natural disturbances in forest ecosystems of Europe, which can damage not only individual trees or groups of trees but also completely destroy forest stands on large areas. In November 2004, forest stands in the Tatra National Park (TANAP) in Slovakia (Central Europe) on an area of 12,000 ha were seriously damaged by northern winds with gusts over 200 km h⁻¹ (Fleischer & Homolová 2011). Ten years later (in 2014), a windthrow again destroyed forest stands. Moreover, frequency and intensity of such disturbances is expected to increase, and temperate forests may experience growing damages in the future (Hlášny et al. 2014). Data from long-term monitoring plots can be very useful for answering the questions about the impacts of climate change on forest ecosystems and the feedbacks between forest ecosystems and climate (Clarke et al. 2011; Margesin et al. 2014).

Windthrow in the Tatra Mts. in 2004 offered an ideal opportunity to study and monitor changes in all landscape components, especially forest ecosystems on the affected area. Therefore, long-term research plots were established in 2005 to facilitate international and interdisciplinary comparative research and monitoring of abiotic and biotic components at plots (Fleischer & Homolová 2011). After...
windthrow the missing crown canopy triggers changes in micro- or meso-climate due to a greater input of precipitation, solar radiation, heat input to the soil surface and a more intense air circulation, followed by changes in the herb layer cover and composition. Due to changed environmental conditions, changes in soil properties are also expected to occur, especially in surface organic layer and the topsoil.

Consequently, abiotic forest disturbances influence habitat conditions for living soil organisms (Ulanova 2000; Certini 2005; Holden & Treseder 2013). The disturbance effect on soil microorganisms persists until aboveground vegetation re-grows and later succession of vegetation can reverse changes in soil properties (Holden & Treseder 2013). Long-term research plots at the windthrow-affected areas in TANAP have enabled monitoring not only of abiotic environments, but also vegetation, fauna and soil organisms. Among soil organisms the attention has been paid especially to soil nematodes, Collembola communities and soil microorganisms (Čerevková & Renčo 2009; Gömöryová et al. 2011; Čuchta et al. 2012; Čerevková et al. 2013; Čuchta et al. 2013; Urbanovičová et al. 2014).

Significant changes in the first months and years after the event are expected mainly for soil properties exhibiting a high temporal variability, such as soil moisture and temperature, soluble nutrient contents, soil organism abundance and activity, etc. Therefore, we hypothesize that windthrow plots and intact forest differ in microbial activity because of different microclimate and organic matter input. The same applies to plots differing in the management regime (plot with extracted fallen trees, plot after fire and plot with fallen trees left in situ), consequently having different microclimate and vegetation species composition. Objectives of this study are: (1) comparing the responses of soil microorganisms to different management regimes on disturbed areas, and (2) evaluating the trends in microbial community size and activity changes during the period 2006 – 2013. Such research is expected to improve our understanding of microbial processes recovery after windthrow.

2. Material and methods

2.1. Site description

The study was performed at four research plots established on windstorm-affected slopes by the Research Station of the TANAP immediately after windthrow and/or fire: a/ REF – reference plot, where spruce stand was not affected by windthrow; b/ EXT – windthrow plot with extracted fallen trees; c/ FIR – windthrow plot with extracted fallen trees, damaged by surface wildfire 6 months after the windstorm; d/ NEX – windthrow plot, where fallen trees were not extracted and the plot was left to undergo the spontaneous succession.

All plots are situated on south- to south-eastern slopes at the elevation of 1,000 – 1,250 m a.s.l. with slope of 5 – 10%. The predominant soil type is Dystric Cambisol formed from glacial moraine deposits with up to 10 cm thick surface organic layer. At the FIR, forest floor was completely burned in 2005, but the mineral soil was not affected by fire.

The intact Norway spruce (Picea abies Karst.) stand with an admixture of larch (Larix decidua Mill.) at the REF plot is >120 year old. In the ground-layer vegetation, mosses, Vaccinium myrtillus and Avenella flexuosa are the most abundant species. The vegetation composition did not change significantly during the observation period. At the EXT plot Calamagrostis villosa, C. arundinacea and Chamaenerion angustifolium are the dominant species and their abundance have not changed distinctly during observed period. Aboveground living and dead organic matter was completely burnt down at the FIR plot. During the following years, C. villosa, C. arundinacea and C. angustifolium spread rapidly and covered a major part of the FIR plot. At the NEX plot, small changes have occurred in comparison to standing forest, later light-demanding herbs and grasses occurred in a mosaic pattern in gaps.

2.2. Soil sampling and sample preparation

Soil samples were collected at 10 m intervals along 90 m long transects located in the central part of study plots, from the mineral A horizon (depth 3 to 10 cm) 1 to 3 times during each vegetation period since 2006. Usually 10 samples were taken from each plot except the summer 2006, and autumn 2011 and 2012, when only 3 to 5 samples per plot were taken. After coarse material and plant roots were hand-removed, a part of the samples were stored in field-moist condition at 4°C prior to microbial analyses. The other part used for chemical analyses was air-dried.

2.3. Laboratory analyses

In air-dried soil samples, soil acidity, organic C and total N were determined. Soil acidity (pH/KCl) was measured potentiometrically in 1 M KCl suspension. VarioMacro CNS Analyser was used to determine soil organic C and total N content.

In fresh soil samples soil water content (SWC) was estimated gravimetrically by oven-drying soil at 105°C for 24 h. Basal soil respiration (BR) was measured by estimating the amount of CO₂ evolved during incubation of 50 g soil in a closed jar for 24 h (Alef 1991). CO₂ absorbed in a 0.05 M NaOH was determined by the titration with 0.05 M HCl using the phenolphthalein indicator. For the assessment of substrate-induced respiration (SIR), glucose was added to soil samples and CO₂ evolved was measured as described above after 4.5 h. Soil microbial biomass C (Cmic) was estimated using the microwave-irradiation procedure according to Islam & Well (1998). C content in the extract was quantified by the oxidation with K₂Cr₂O₇/H₂SO₄ and titrimetrically by (NH₄)₂Fe(SO₄)₂. N – mineralisation (Nmin) was determined using the anaerobic incubation according to the procedure described by Kandeler (1993). Catalase activity (Acat) was measured 10 min after 3% H₂O₂ was added to soil sample. The measurement is based on the volume of discharged oxygen based on the method of Khaziev (1976). The community-level metabolic profiles of soil microbial community were estimated using BIOLOG EcoPlates (Insam 1997). Inocula were produced by resuspending fresh soil in 0.9% NaCl,
supernatant was diluted 1:10,000, and 150 ml of extract were incubated in microtitration plates at 37°C during 7 days. Absorbance at 590 nm was recorded using the Sunrise Microplate Reader (Tecan, Salzburg, Austria). Metabolic activity was calculated as the area below the time-absorbance curve, and was used as a measure of the abundance of the respective functional group.

2.4. Data analyses

All the results were expressed on the oven-dry basis. The richness of the soil microbial community was assessed as the number of substrates with absorbance exceeding 0.2. The functional diversity of the microbial community was assessed using Hill’s $N_2$ diversity indices (Hill 1973):

$$N_2 = 1/\Sigma p_i^2$$

where $p_i$ is the frequency of the $i$-th functional group.

Microbial activity data were treated by the analysis of covariance with season and plot as fixed categorical factors, observation year as a fixed factor nested within season, and soil moisture as a continuous covariate. Pairwise contrasts were tested using Tukey-Kramer tests. To assess overall temporal trends, microbial characteristics were regressed against observation dates (expressed as the number of days after January 1, 2006 when soil sampling was done). All statistical analyses were performed using the GLM procedure of the statistical package SAS/STAT® (SAS 1988).

3. Results

3.1. Differences in microbial community between plots

Soil microbial characteristics varied significantly during observed period at study plots (Table 1). Generally, richness and diversity of functional groups exhibited the highest variability. Basal and potential respiration varied less than microbial biomass and especially N–mineralisation. While the differences in variability of N–mineralisation and catalase activity between plots are small, the variability of the other characteristics differed much more. Basal and potential respiration varied more in the forest stand (REF) than at the extracted plots (EXT and FIR). Surprisingly, the highest variability in richness and diversity was found at the FIR plot; at the other plots the differences in variability were small.

| Table 1. Coefficients of variation (%) of soil microbial attributes at the study plots. |
|---|---|---|---|---|---|---|---|---|
| REF | EXT | FIR | NEX |
| Basal respiration (BR) | 63.85 | 56.70 | 51.01 | 60.19 |
| Substrate-induced respiration (SIR) | 65.99 | 56.99 | 51.31 | 50.84 |
| Microbial biomass C (Cmic) | 59.43 | 70.22 | 68.27 | 66.25 |
| N–mineralisation (Nmin) | 68.63 | 74.41 | 72.32 | 71.84 |
| Catalase activity (Acat) | 57.53 | 49.57 | 56.81 | 54.58 |
| Richness | 127.68 | 126.88 | 136.35 | 128.24 |
| Diversity | 128.38 | 125.51 | 136.46 | 129.83 |

The analysis of covariance of soil microbial characteristics based on a nine-year-long monitoring showed that there are significant differences in most of soil microbial characteristics between years, seasons and plots (Table 2). There are some exceptions – microbial biomass did not differ between seasons, and interestingly, no significant differences in richness and diversity between plots were detected.

| Table 2. Analyses of covariance of microbial community attributes (significances of F-tests). |
|---|---|---|---|---|---|---|---|---|
| Season | *** | *** | *** | ns | *** | *** | *** |
| Plot | *** | *** | *** | *** | ns | ns | ns |
| Season × plot | I* | ns | ns | *** | *** | ns | ns |
| Year × season | *** | *** | *** | *** | *** | *** | *** |
| SWC | *** | *** | *** | *** | *** | ns | ns |

Significance labels: *** P<0.001, ** P<0.01, * P<0.05, ns – non-significant.

Generally, microbial activity was higher at the reference and fire plots in comparison to the extracted and non-extracted plots (Figures 1a–e). Although we expected that different management regimes will be reflected in soil microbial characteristics, the results indicate that there are no significant differences between the extracted and non-extracted plots. On the other hand, this pattern is not uniform among years as indicated by significant season × plot interactions for most microbial characteristics, except of catalase activity.

3.2. Changes of microbial community characteristics during the years 2006 – 2013

To find out if there is any trend in the increase or decrease of microbial activity at study plots during observed period we tested the significance of the linear regression (trend) between microbial characteristic and years. Among microbial characteristics only potential respiration, microbial biomass C and N–mineralisation showed a significant linear trend (Table 3) with a slight increase observed at all windthrow-affected plots. On the other hand the changes in basal respiration, catalase activity and richness and diversity were more fluctuating and no distinct trend was observed.

| Table 3. Significance of temporal trends of microbial community attributes (significances of F-tests) during the observation period. |
|---|---|---|---|---|---|---|---|
| REF | D* | ns | ns | ns | ns | ns | ns |
| EXT | ns | ns | I** | I* | I* | ns | ns |
| FIR | ns | ns | I* | | I* | ns | ns |
| NEX | ns | ns | I* | I** | I** | ns | ns |

Significance labels: *** P<0.001, ** P<0.01, * P<0.05, ns – non-significant; I – increasing trend, D – decreasing trend.

4. Discussion

The responses of soil microorganisms to natural and anthropogenic pressures or stresses on soil ecosystem are very quick due to their high surface-to-volume ratio, causing that they are capable of a much more intense exchange of matter and energy with their environment (Nielsen & Winding 2002). Therefore we supposed that soil microbial community will differ between different plots as well as between the plot...
carbon are often reduced because of losing above- and below-ground sources (needles, leaves, root exudates) what can be reflected by decreased microbial activity.

The analysis of covariance showed a significant effect of soil water content on most microbial attributes. However, the results from an earlier study (Gömöryová et al. 2011) showed that the response of soil microbial attributes to C content were more distinct than those related to soil water content; in addition, a positive relationship was found between soil organic carbon content and microbial activity. Unexpectedly, there are no significant differences between the EXT and NEX plots in spite of the fact that they differ in the above-ground biomass and microclimate. We suppose that soil microbiota was stressed after opening of the canopy considerably in comparison to the reference plot. On the other hand, a thick surface organic layer at both NEX and EXT plot has isolating and buffering effect on mineral soil and consequently on soil microbial communities from the above-ground microclimate and herb layer changes at these plots. This may be the reason why no significant differences between the EXT and NEX plots have been identified yet.

Most studies showed that soil microbial biomass after fire decreases as surface temperature can reach up to 600°C during with standing trees and closed canopy and the plots without canopy; the latter also differ from each other distinctly (the plot with extracted trees, the burnt plot, the plot with fallen trees left in situ). However, our expectations were fulfilled only partially. The highest microbial activity and biomass were observed at the REF and FIR plots, which are very contrasting localities. In contrast, these indicators were lowest at the EXT and NEX plots. In the case of intact forest this can be explained by the fact that soil microorganisms were exposed to smaller environmental stress than the windthrow-affected ones (sudden canopy opening leads to bigger fluctuations of soil temperature, moisture, etc.). In the case of FIR, higher microbial activity may be related to nutrients released from the burnt organic material (Gömöryová et al. 2011). Similarly different responses of soil microorganisms to the opening of canopy due to windthrow or harvesting were observed by Holmes & Zak (1999), Barcenas-Moreno et al. (2009) or Holden & Treseder (2013). On one hand, abiotic disturbance can initiate higher microbial activity due to increased solar radiation, heat input to soil surface and better air circulation. On the other hand, openings can be associated with higher soil moisture due to the absence of water losses through transpiration and interception. Moreover, sources of labile carbon are often reduced because of losing above- and below-ground sources (needles, leaves, root exudates) what can be reflected by decreased microbial activity.

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fire. Fire can also remove large amounts of organic C, including labile components (Certini 2005; Holden & Treseder 2013). We suppose that the surprisingly high activity and biomass at the FIR plot could be explained by better living conditions for soil microorganisms. Fire damaged the surface organic layer but not the A-horizon. A thick humus layer was burnt and reduced, and the released mineral nutrients could have enriched the underlying A-horizon. A missing difference in abundance and diversity of microbial functional groups between plots, however, does not imply that there are no differences in microbial community composition. BIOLOG EcoPlates contain only 31 carbon substrates; they do not necessarily reflect the status of the whole microbial community. In most studies it was shown that harvesting and fire altered the composition of microbial communities and that microbial species are differently affected by disturbance (Certini 2005; Barcenas-Moreno et al. 2011). However, the functional consequences of microbial compositional changes require further testing.

The season × plot interaction was significant, which means that differences between plots are not consistent throughout the year. Moreover, there is a high within-plot spatial variability (49–136%) of microbial attributes, which complicates the comparison of data. We expected the highest variability at the REF and NEX plots as standing or fallen trees do not enable uniform distribution of precipitation or heat on the soil surface. However, this was true only partially for basal and potential respiration in the REF stand. The highest within-plot variability of microbial functional-group richness and diversity at the FIR plot could result from differences in burning intensity across the study plot.

Monitoring of soil properties, including microbial characteristics, has been going on for nine years after the windthrow in the Tatra Mts. This is quite a short time in view of forest longevity. However, mesoclimates have completely changed and also rapid changes in the vegetation due to succession have occurred. Therefore, we expected to observe smooth increasing or decreasing trends in microbial biomass, activity or composition of microbial community. The first years after the windthrow, the herb layers at the REF and NEX plots were very similar, later at the NEX plot *Calamagrostis* appeared in gaps. Immediately after the windthrow and fire, extracted plots differed in plant cover because at the FIR plot the herb layer was destroyed, but during the following vegetation seasons *C. angustifolium* and *C. villosa* covered a major part of the FIR plot, and plant communities on the FIR and EXT plot converged. The results showed that in contrast to the standing forest, signs of a temporal trend can be detected at the windthrow plots. A gradual recovery of microbial community can thus be expected. Significant changes with time were found for microbial biomass, SIR and N-mineralisation. This is consistent with the meta-analysis by Holden & Treseder (2013). They showed that a soil microbial biomass decline was observed following disturbances in many studies and a significant positive relationship exists between the time since disturbance (fire and harvesting) and the microbial biomass recovery. Their finding suggests that forest disturbance can have long-term consequences for belowground communities and the recovery needs at least 10–15 years following the disturbance. Inconsistency of temporal trends among microbial characteristics can be associated with the methodology, as they were generally not assessed in situ but under optimized laboratory conditions (Dilly et al. 2003; Margesin et al. 2014).

5. Conclusions

The presented study showed that differences occur in microbial activity and biomass at the disturbed plots in comparison to an intact forest. Generally, the highest microbial activity and biomass were observed at the REF and FIR plots. At the REF plot this is probably due to the fact that soil microorganisms were not exposed to stress. At the FIR plot it can be associated with the reduction of thick surface organic layer after burning and a following enrichment of the A-horizon by released nutrients. Surprisingly, no significant differences in microbial characteristics were found between as different plots as the extracted and non-extracted plot. The present results indicate that at higher altitudes, the effect of mesoclimatic is more important than the differences in microclimate due to different management. However, we cannot exclude methodological limits, as the plots were established without replication. Our results also show slight recovery trend of microbial community at windthrow affected plots.

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