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Impact of wildfire intensity and logging on fungal and nitrogen-cycling bacterial communities in British Columbia forest soils

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ABSTRACT

Wildfire and logging are common disturbances in the forests of northwestern North America, causing changes in soil chemistry and microbiology, including fungal and nitrogen-cycling bacterial communities. These organisms play key roles in nutrient cycling, and affect the regeneration of tree seedlings after disturbance. We studied the effects of wildfire and logging on fungal and nitrogen-cycling communities in the rhizosphere of 16 month-old Douglas-fir seedlings as they regenerated in burned and logged soils. Seeds were planted against root windows that were set up vertically in the soil, with a removable front panel used to access the seedling rhizosphere soil surface. Windows were established in control, lightly burned, and severely burned plots, as well as two types of logged plots (clearcut and screefed clearcut). Soil scrapings from the root window–soil interface were taken and the structure of fungal and nitrogen-cycling communities was resolved using length-heterogeneity PCR (LH-PCR) of fungal nuclear ribosomal RNA genes, and terminal restriction fragment length polymorphism (T-RFLP) analysis of *nifH* and *nosZ* genes. We found striking differences in the community structure of fungal, denitrifying, and N-fixing communities in response to burning and logging. With the exception of clearcut and screefed clearcut, which were generally similar, each treatment had a unique impact on community structure for these genes. Burning and logging also impacted the relative richness and evenness of these communities. Fungal relative richness and evenness increased in response to logging and severe burning, while denitrifier relative richness and evenness increased in all disturbance treatments, and N-fixing bacterial relative richness and evenness decreased in response to burning. The greatest differences in microbial community structure, relative richness, and evenness were found in the comparisons of lightly burned and logged treatments. The results suggest that the presence of an intact forest floor influences soil microbial communities less than the presence of living trees.

1. Introduction

Temperate and sub-boreal forests are subject to natural and man-made disturbances such as wildfire, insect infestations, and clearcutting. The extent and severity of these disturbances are likely to increase as climate change causes higher temperatures and increased drought (Hamann and Wang, 2006). Undisturbed forest communities tend to be biologically stable, with indigenous microbial populations maintaining balanced nutrient cycles (Paul and Clark, 1989). Disturbances such as fire and clearcutting alter both aboveground forest properties (removal of trees and soil) and belowground soil chemistry, microbial communities, and nutrient cycling (Ballard, 2000; Bárcenas-Moreno and Bååth, 2009; Goodale and Aber, 2001; Jiménez Esquilin et al., 2008; Yeager et al., 2005). These changes can affect the regeneration of ecologically and commercially valuable tree species such as Douglas-fir (*Pseudotsuga menziesii*). In disturbed areas, interactions between seedling roots and soil microbes may be crucial to seedling survival. Colonization of seedling roots by ectomycorrhizal (EcM) fungi can provide greater access to nutrients and water (Schoonmaker et al., 2007) and promote carbon cycling (Talbot et al., 2008). Bacteria associated with EcM are known to mobilize key nutrients from minerals and organic substrates (Burke et al., 2008; Frey-Klett et al., 2007), while many key processes, such as nitrogen fixation, can be carried out by bacteria living freely in the soil (Izquierdo and Nüsslein, 2006).

Fungi account for the bulk of microbial biomass in forest soils, and play major roles in nutrient-cycling processes either as free-living saprotrophs or as mycorrhizae (Dighton, 2003). EcM fungi, in particular, play a pivotal role in Douglas-fir seedling establishment (Simard, 2009; Twieg et al., 2007). Fire has been demonstrated to alter the community structure of both soil fungi (Bárcenas-Moreno and Bååth, 2009; Cairney and Bastias, 2007) and EcM fungi

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(Dahlberg et al., 2001; Smith et al., 2004). Similarly, clearcut logging can alter forest fungal composition, with different EcM fungi found in clearcuts compared to undisturbed forests (Jones et al., 2003).

Nitrogen is typically the limiting nutrient in northern forest soils (Widmer et al., 1999). Burning and logging cause long-term changes in N-cycling (Goodale and Aber, 2001), affecting the size of N pools in soils (Grogan et al., 2000; Neary et al., 1999; Wan et al., 2001) and the composition of N-cycling microbial communities (Reich et al., 2001; Shaffer et al., 2000; Walley et al., 1996). Nitrogen fixation is carried out by a wide range of heterotrophic bacteria, but all have in common the gene for nitrogenase reductase (*nifH*), making it an ideal molecular marker (Zehr et al., 2003). Denitrifying bacteria are a similarly heterogeneous group, but focusing on the gene encoding nitrous oxide reductase (*nosZ*) allows detection of organisms that are capable of completing the denitrification cycle (Throbäck et al., 2004).

In recent years, molecular advances have allowed ecologists using techniques such as length-heterogeneity polymerase chain reaction (LH-PCR) and terminal restriction fragment length polymorphism (T-RFLP) to quickly profile microbial populations in the environment (Thies, 2007). These "fingerprinting" techniques provide a powerful way to gain information about the relative richness of the targeted gene (number of genotypes), while the fragment peak height gives some indication of the relative abundance of each genotype within a soil sample. By targeting functional genes, DNA-based community structure can be linked to potential ecosystem function. Although the extent of the relationship between genetic community structure and physiological function has not yet been proven (Wallenstein et al., 2006), there is evidence of coupling between the two (Kandeler et al., 2006; Rich et al., 2003), and analysis of functional genes remains a useful method for investigating changes in specific functional communities.

In the summer of 2003, large portions of the southern BC interior were burned by forest fire. This provided a unique opportunity to study the effect of wildfire on microbial communities. In order to separate the effects of burning from those of manual tree removal and forest floor reduction, we planted interior Douglas-fir (*P. menziesii* var. *glauca* (Beissn.) Franco) seeds in areas burned to different severities, established forests, clearcuts, and screefed clearcuts. We examined shifts in the community structure of soil fungi using LH-PCR, and of nitrogen-fixing and denitrifying microbes using T-RFLP analysis of *nifH* and *nosZ* genes, respectively.

2. Materials and methods

2.1. Site descriptions

The study was established over an 850 km^2 area in the Cascade Dry, Cool variant of the Interior Douglas-fir biogeoclimatic zone, near the town of Barriere, British Columbia (51.12N, 120.07W). This was where the McLure fire burned 26,000 ha of forest in the summer of 2003. The 12 sites were circum-mesic in soil moisture regime and ranged in elevation from 806 to 1268 masl. The original canopy at all sites was dominated by interior Douglas-fir, with minor components of lodgepole pine (*Pinus contorta* var. *latifolia*) (Stark et al., 2006). Sites classified as 'high-severity burns' had been exposed to a stand-destroying wildfire, with all needles on the trees consumed during the fire. At these sites, it was apparent from the exposed mineral soil that most of the forest floor had been consumed (0.7 ± 0.2 cm depth compared to 4.9 ± 0.6 cm in control mature forests). The remaining burned trees on these sites were salvage logged over the winter and early spring of 2004, before the initiation of our study. Sites classified as 'low-severity burns' were exposed to surface-level fire. At these sites, at least 80% of the trees survived immediately after the fire. The understory vegetation was consumed and there was evidence that a good portion of the forest floor was lost during the fire (1.8 ± 0.5 cm). The clearcut sites, which had been logged in the summer of 2003, contained two types of forest floor disturbance: none (3.8 ± 0.4 cm depth) or with the forest floor removed to expose mineral soil (0 cm of forest floor). The clearcuts and control mature forests were located either within the overall perimeter or at the immediate periphery (within 4 km) of the McLure fire.

2.2. Experimental design

The study was established as a completely randomized design with three replicate sites of each of four disturbance treatments: control forests, clearcuts, forests disturbed by low-severity burns, and forests disturbed by high-severity burns. Clearcut sites had two types of plots: those with the forest floor left undisturbed and those with the organic horizons removed (referred to as 'screefed clearcut'). In order to access the root/soil interface, three root windows (transparent acrylic panel (77 cm × 52 cm × 0.6 cm) with a 30 cm × 30 cm trap door (Dong et al., 2007) were installed at each plot. Douglas-fir seed (Seedlot 48523 from the British Columbia Ministry of Forests Tree Seed Centre, Surrey, B.C.) was moist-stratified at 4 °C for 3–6 weeks. From late May to mid June 2004, seeds were planted 1 cm apart in 5-mm deep depressions, as close to the window as possible (Jones et al., 2010). Pits used to access the root windows were covered throughout the experiment to maintain light levels and temperature comparable to soil conditions.

2.3. Sampling

Soil samples were collected from the root window interface in October 2005 using a metal grid. The 20 cm × 20 cm square grid was composed of sixteen 5 cm × 5 cm squares. The top of the grid was matched to the top of the forest floor at each window, and light scrapings of soil (approx. 5 g) from each square were taken using wooden sticks. As the upper 5 cm of forest floor was often composed of litter, or eroded, only the scrapings from the 8 squares in the middle 5–15 cm of the grid were used, for a total of 72 soil samples for each treatment. The grid was placed on the vertical surface of the root window. The soil was collected into plastic bags and kept at 4 °C while transported back to the laboratory, where it was frozen at −20 °C until DNA extraction.

2.4. Soil chemical analysis

The soil mineral layer was sampled in September 2004 (Jason Barker, University of British Columbia, personal communication). The top horizons of forest floor were removed to expose mineral soil. Using a hand held soil corer, five sub-samples per site were taken, combined, and sent to the British Columbia Ministry of Forests, Research Branch Analytical Laboratory in Victoria, British Columbia. Soils were analyzed for total C, available P (Bray) and pH (water) according to Kalra and Maynard (1991). Mineralizable N (as NH_4-N) was determined using a 2-week anaerobic incubation (Bremner, 1996).

2.5. DNA preparation

DNA from all soil samples was extracted using Ultraclean Soil DNA kits (MoBio, Carlsbad, CA, USA), following the manufacturer's recommended alternative protocol for increased yield.

2.6. PCR amplification of the fungal community (LH-PCR)

A segment of the fungal rDNA intergenic spacer region containing part of the 5.8S rRNA gene, the second internal transcribed