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Change in soil fungal community structure driven by a decline in ectomycorrhizal fungi following a mountain pine beetle (*Dendroctonus ponderosae*) outbreak

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Summary

- Western North American landscapes are rapidly being transformed by forest die-off caused by mountain pine beetle (*Dendroctonus ponderosae*), with implications for plant and soil communities. The mechanisms that drive changes in soil community structure, particularly for the highly prevalent ectomycorrhizal fungi in pine forests, are complex and intertwined. Critical to enhancing understanding will be disentangling the relative importance of host tree mortality from changes in soil chemistry following tree death.
- Here, we used a recent bark beetle outbreak in lodgepole pine (*Pinus contorta*) forests of western Canada to test whether the effects of tree mortality altered the richness and composition of belowground fungal communities, including ectomycorrhizal and saprotrophic fungi. We also determined the effects of environmental factors (i.e. soil nutrients, moisture, and phenolics) and geographical distance, both of which can influence the richness and composition of soil fungi.
- The richness of both groups of soil fungi declined and the overall composition was altered by beetle-induced tree mortality. Soil nutrients, soil phenolics and geographical distance influenced the community structure of soil fungi; however, the relative importance of these factors differed between ectomycorrhizal and saprotrophic fungi. The independent effects of tree mortality, soil phenolics and geographical distance influenced the community composition of ectomycorrhizal fungi, while the community composition of saprotrophic fungi was weakly but significantly correlated with the geographical distance of plots.
- Taken together, our results indicate that both deterministic and stochastic processes structure soil fungal communities following landscape-scale insect outbreaks and reflect the independent roles tree mortality, soil chemistry and geographical distance play in regulating the community composition of soil fungi.

Introduction

Soil fungi form diverse and spatially variable communities (Talbot *et al.*, 2014; Tedersoo *et al.*, 2014), where mycorrhizal and saprotrophic fungi play fundamental roles in the cycling of nutrients (Cairney & Meharg, 2002; Lindahl *et al.*, 2007). Mycorrhizal fungi acquire carbon from and enhance the water and nutrient uptake of living host plants (Smith & Read, 2008) and decompose soil organic material (Shah *et al.*, 2016). Saprotrophic fungi decompose plant litter and wood to acquire carbon and other nutrients, as well as mobilize nutrients from soil organic material (Cairney & Meharg, 2002; Floudas *et al.*, 2012). The mechanisms by which communities of different groups of soil fungi are assembled may vary as a consequence of differences in their trophic lifestyles (Peay *et al.*, 2013, 2015; Kivlin *et al.*, 2014). Disturbances that result in the death or removal of host trees such as wildfire (Dahlberg, 2002), harvesting (Barker *et al.*, 2013) and insect-caused mortality (Del Vecchio *et al.*, 1993; Saravesi *et al.*, 2015) may affect ectomycorrhizal and saprotrophic fungi differently, as they rely on living and dead plants, respectively. For instance, tree death typically changes the community structure of ectomycorrhizal fungi through reductions in richness and abundance, while the richness of saprotrophic fungi has been shown to increase with a shift in composition following tree mortality (Stursova *et al.*, 2014; Treu *et al.*, 2014). Environmental conditions such as soil chemistry, soil moisture, soil nutrients,

and substrate quality can alter community composition and diversity of both groups of fungi (Egerton-Warburton & Jumpponen, 2005; Toljander *et al.*, 2006; Prescott & Grayston, 2013; Ottosson *et al.*, 2014; Treseder *et al.*, 2014). The death or removal of a host tree often coincides with changes in soil environmental conditions, and thus disentangling the relative importance of tree mortality from changes to soils following tree death is difficult, particularly with regard to ectomycorrhizal fungi which functionally connect trees and soils.

In addition to differences in the relative importance of deterministic factors governing fungal community assembly, the mechanism(s) of assembly by which these communities respond can also differ based on stochastic fluctuations in species immigration in combination with dispersal limitation (Cline & Zak, 2014; Crowther *et al.*, 2014; Bahram *et al.*, 2016). Assembly of fungal communities has been observed not to be connected to measured indicators of the local environment, for example, in the upper surface layers of soil (10–30 cm) (Powell *et al.*, 2015) as well as on roots of host plants (Beck *et al.*, 2015). Furthermore, the community composition of saprotrophic fungi has been found to be more dissimilar with increasing distance between samples (Feinstein & Blackwood, 2013; Tedersoo *et al.*, 2014). However, recent evidence suggests that both deterministic and stochastic processes work in tandem to regulate the assembly of ecological communities (Chase & Myers, 2011; Hanson *et al.*, 2012; Feinstein & Blackwood, 2013). For example, Dumbrell *et al.* (2010) found that, although soil abiotic factors, particularly soil pH, were strong predictors of mycorrhizal fungal community composition and diversity, these communities were also influenced by dispersal limitation (Dumbrell *et al.*, 2010). Shifts in saprotrophic fungal composition and diversity have also been observed to be governed by both deterministic and stochastic processes, for example, as shown on decomposing leaves from the O horizon of upland and riparian forests (Feinstein & Blackwood, 2013) and from remaining tropical forests on former agricultural and logged areas of Puerto Rico (Bachelot *et al.*, 2016). Nonetheless, it is critical to determine the extent to which soil fungi are constrained by deterministic or stochastic processes, as these taxa are pivotal to ecosystem functions such as carbon and nutrient cycling as well as forest regeneration and succession (Jones *et al.*, 2003; Read & Perez-Moreno, 2003; Clemmensen *et al.*, 2013).

In western Canada, the mountain pine beetle (*Dendroctonus ponderosae*), a native of temperate conifer forests in western North America, has expanded east of the Rocky Mountains into pine habitats reaching as far as the boreal forest (Cullingham *et al.*, 2011). Our previous research in this region has shown that, compared with undisturbed stands, beetle-killed lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) stands have higher soil moisture content, higher soil nutrient availability and decreased concentrations of soil phenolics (Cigan *et al.*, 2015). Soil phenolics, a broad class of carbon-rich plant secondary compounds, are known to influence nutrient cycling, particularly the availability of nitrogen (N) (Northup *et al.*, 1995; Hättenschwiler & Vitousek, 2000). Increased concentrations of soil phenolics have led to decreased colonization rates in ectomycorrhizal fungi (Siqueira *et al.*, 1991), shifts in the community composition of fungi on the roots of their hosts (Krupa & Fries, 1971), and positive to negative responses in spore germination and hyphal growth for soil fungi (Kuiters, 1990; Siqueira *et al.*, 1991; Bárta *et al.*, 2010). Our previous work has demonstrated a decrease in the richness of fruiting bodies of ectomycorrhizal fungi in beetle-killed compared with undisturbed pine stands (Treu *et al.*, 2014) as well as compositional differences in fungal communities on lodgepole pine seedlings that were inoculated with soil fungi from undisturbed and beetle-killed stands in a glasshouse experiment (Karst *et al.*, 2015).

In this study, we build on this previous research (Treu *et al.*, 2014; Karst *et al.*, 2015) to report changes in the richness and community composition of soil fungi following a landscape-scale beetle outbreak. We used next-generation sequencing of DNA from soil fungi together with measurements of tree mortality, soil abiotic factors (i.e. nutrients, moisture, and phenolics) and geographical distance between samples. We used this information to answer the following questions with regard to the overall soil fungal community and specific functional groups, that is, ectomycorrhizal or saprotrophic fungi. Do increases in tree mortality result in decreases in the richness of soil fungal communities with subsequent effects on community composition? How does variation in soil nutrients, moisture, and phenolics influence the richness and composition of soil fungi? What is the relative importance of tree mortality, soil abiotic factors, and geographical distance between samples in determining the composition of soil fungi?

Materials and Methods

Study area

Eleven forest stands were located within a 625-km^2 region experiencing mountain pine beetle (*Dendroctonus ponderosae* Hopkins) activity since 2009 and bordering provincial permanent sampling plots within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta (54°39′N, 118°59′W; 950–1150 m above sea level). Canopies were dominated (≥ 80%) by even-aged (mean ± SE: 120 ± 0.4 yr old) lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var. *latifolia* Engelm.) and across stands, a gradient of beetle-induced tree mortality was captured (0–82% lodgepole pine basal area killed) (Cigan *et al.*, 2015). Although trees were not explicitly tested, we infer that they are lodgepole pine and not lodgepole pine × jack pine (*Pinus banksiana* Lamb) hybrids based on genetic ancestry maps (Cullingham *et al.*, 2012). Within stands, *Abies balsamea* (L.) Mill, *Betula papyrifera* Marshall, *Picea glauca* (Moench) Voss, *Picea mariana* Mill. Britton, Sterns, & Pogenb., and *Populus tremuloides* Michx. were interspersed in the subcanopy (0–14% of total basal area) along with a mixture of mostly herbaceous (e.g. *Chamerion angustifolium* (L.)) and to a lesser extent woody (e.g. *Vaccinium* spp.) vegetation in the understory (Pec *et al.*, 2015). Soils were classified as Orthic Gray Luvisols derived from imperfectly drained glacial tills (Soil Classification Working Group, 1998). The stands were typical of lodgepole pine forests found within this region of Alberta (Beckingham *et al.*, 1996), exhibiting

similar initial stand productivity (Treu *et al.*, 2014) and edaphic characteristics while showing no relationship to any topographic characteristics such as elevation, slope or aspect (Cigan *et al.*, 2015). Detailed information on stand selection and description, including stand locations and structure, is presented in Treu *et al.* (2014) and Cigan *et al.* (2015).

In May 2012, we installed a 1600-m^2 (40 × 40 m) plot within each of the 11 stands. Within each plot in June 2012, 10 soil cores (5 cm diameter; 20 cm deep) were sampled for fungi found on fine roots and in soil at a distance of 1 m from the nearest mature pine tree. A minimum distance of 7 m was maintained between sampled soil cores to increase sample independence (Pickles *et al.*, 2012). Geographical coordinates (Garmin GPSmap 60Cx; Garmin International, Olathe, KS, USA) were also recorded at each sampling point. To determine the effect of tree mortality on fungal richness and composition, we recorded diameter at breast height (≥ 1.3 m), species identity, and health status (i.e. alive or dead, crown color and years dead), as described by Klutsch *et al.* (2009), of all mature pine trees within a 3-m radius of each sampled soil core in June 2012. We also measured the diameter at breast height and species identity of all subordinate tree species within this same 3-m radius. Attack by mountain pine beetle on mature lodgepole pine trees was confirmed by the presence of pitch tubes, boring dust, exit holes, and subcortical galleries (Safranyik & Carroll, 2006). Tree mortality was calculated as lodgepole pine basal area killed over total basal area of all trees, expressed as a percentage.

To determine whether variation in soil moisture, nutrients, or phenolics was associated with changes in fungal richness and composition, these factors were measured within 20 cm of each sampled soil core. In brief, we sampled supply rates of macronutrients (i.e. calcium (Ca), potassium (K), magnesium (Mg), N, phosphorus (P) and sulfur (S)) using Plant Root Simulator (PRS™) probes (Western Ag Innovations, Inc., Saskatoon, SK, Canada). Probes were inserted 10 cm (length of probe) into mineral soils of the A-horizon in June 2012, removed from the soil in August 2012, cleaned, and shipped to Western Ag Laboratories for macronutrient analysis. Volumetric soil moisture content was measured from the upper 10 cm of the soil column using a Theta Probe soil moisture sensor (Delta-T Devices, Cambridge, UK) during June 2012. To determine phenolics, soil samples from the top of the A-horizon were taken within 2 wk of soil core sampling in early July 2012. Samples were air-dried for 48 h and a quantitative assay for phenolic acids and compounds was used (Tel & Covert, 1992). We added Folin–Ciocalteau phenol reagent (Sigma-Aldrich, Oakville, ON, Canada) to soil water extracts followed by spectroscopy (Enesys 10S UV-vis Spectrophotometer; Thermo, Fisher Scientific, Madison, WI, USA) to determine absorbance (750 nm). A more detailed description of the sampling methods is available in Cigan *et al.* (2015) and Pec *et al.* (2015). These measures were part of a more detailed field survey on nutrient cycling dynamics following beetle outbreak and thus measures were taken to be representative of key biogeochemical periods (see Cigan *et al.*, 2015). In the present study, we used these measures to understand the broader indirect impacts of beetle outbreak on soil fungal community structure. Although our soil cores were sampled to a greater depth than those for the soil abiotic factors measured, this depth best represents the location of soil fungi in the soil profile, particularly ectomycorrhizal fungi, and is comparable to sampling depths in other field studies from boreal systems (Pickles & Pither, 2014).

Sampling and molecular characterization of fungi

Fungi occurring in soils and on roots were sampled from the soil cores. In total, 110 samples (10 soil cores × 11 plots) were transported on ice and frozen at −20°C until processed. Soil samples were thawed and fine roots (< 2 mm) as well as any higher order roots were washed and separated using a #14 (1.4-mm opening) sieve stacked over a #80 (180-micron opening) sieve. Subsamples of 125 mg of roots as well as 125 mg of previously subsampled soil (from the same soil core) were placed in a pre-chilled freeze-dryer (VirTis Freezermobile FM25XL; SP Scientific, Warminster, PA, USA) at −45°C and lyophilized for 24 h. Freeze-dried roots were combined with subsampled soil, twice ground and homogenized to a fine powder using a mixer mill (Retsch Type MM 301; Retsch GmbH, Haan, Germany) for 1 min at 25.0 Hz. Genomic DNA was isolated from 250 mg of ground roots and soil using a cetyl trimethlyammonium bromide protocol according to (Roe *et al.*, 2010) with one modification: pellets were resuspended in 50 µl of nuclease-free water (Life Technologies, Carlsbad, CA, USA).

A two-step PCR amplification was performed to amplify the internal transcribed spacer (ITS) 1 region of nuclear rDNA using primers ITS1F and ITS2 and this was sequenced on an Ion Torrent™ PGM 400 Sequencing Kit and Ion 316™ Chips (Life Technologies) at the Molecular Biological Sciences Facility, University of Alberta (see Supporting Information Methods S1 and Table S1). Quality filtering, clustering of sequences, and taxonomic identities of representative sequences were performed through bioinformatic analysis of Ion Torrent™ data using the QIIME pipeline v.1.8 (Caporaso *et al.*, 2010) (see Methods S1). Representative sequences of fungal operational taxonomic units (OTUs) are deposited in GenBank under accession numbers KR584666–KR584685 and KX497205–KX498025.

Statistical analysis

All statistical analyses were carried out using R v.3.1.2 (R Development Core Team, 2015). Sequence data (2 319 302 sequences; min = 305; max = 475 250; mean = 24 159; SE = 7011 across soil cores) were first rarefied to account for uneven sequence depths. As we wanted to directly compare one soil core to another, we rarefied the number of sequences in each soil core to the minimum number of sequences observed across all soil cores (305 sequences), which is a suitable approach used with next-generation sequencing data (Gihring *et al.*, 2012), using 1000 iterations with the RAREFY function in the package VEGAN (Oksanen *et al.*, 2013) (Figs S1, S2). As a consequence of the drastic subsampling of the data based on the uneven sequencing depth across soil cores, of which 70% were below 12 000 sequences, only 1.3% of the available data was used for downstream

analyses. Although 305 sequences per soil core is a low number by high-throughput standards (Smith & Peay, 2014), this sequencing depth was representative of the diversity present (Fig. S2). Richness and composition of soil fungi were calculated at both the community and functional group levels (see Methods S1 and Table S2 for placement of fungi into functional groups).

To test for changes in the richness of soil fungi as a result of beetle-induced tree mortality at both the community and functional group levels, we employed linear mixed effects models using the R package NLME (Pinheiro *et al.*, 2015). Linear mixed effects models were also used to determine if variation in soil abiotic factors was associated with changes in fungal richness at both the community and fungal group levels. Tree mortality, soil nutrients, moisture, and phenolics were included as fixed factors and had low levels of colinearity (Table S3). Site was used as a random factor to account for potential spatial autocorrelation attributable to the clustering of samples into sites. All macronutrients except for Mg were correlated with one another (Table S3). Magnesium was not associated with changes in the richness or composition of soil fungi and was dropped from further analyses. A principal components analysis (PCA) using a correlation matrix was conducted on the following macronutrients: N, K, Ca, P and S. PCA 1 axis scores, which explained 75% of the variation in the data structure, were used to describe soil nutrients in all subsequent analyses. All model assumptions were checked by visual inspection of residual diagnostic plots (Zuur *et al.*, 2009).

Indicator species analysis was performed to identify strongly responding fungal OTUs found across the tree mortality gradient using the MULTIPATT function in the R package INDICSPECIES (Cáceres & Legendre, 2009). To determine if particular groups of taxa were gained or lost across the tree mortality gradient, we calculated a ratio reflecting the number of OTUs gained or lost for a particular phylum or order by dividing the total number of gains by the total number of losses. Values greater than 1 indicated that gains exceeded losses and values less than 1 indicated that losses exceeded gains. Nonparametric *t*-tests with 999 Monte Carlo permutations were used to statistically evaluate the gain and loss of fungal phyla or orders present in soil cores from undisturbed ($n = 23$) compared with severely beetled-killed ($n = 30$) (> 60% *Pinus contorta* killed basal area) stands.

To determine the relative importance of beetle-induced tree mortality, variation in soil abiotic factors (see Table S4 for a detailed summary of soil parameters), and geographical distance in determining fungal composition, we first calculated principal coordinates of neighbor matrices (PCNM) spatial eigenvectors based on geographical coordinates of sampled soil cores using the PCNM function in the R package VEGAN (Oksanen *et al.*, 2013). These PCNM vectors represent spatial variation among sampled soil cores across the study area and are used as explanatory variables suitable for constrained ordination analyses (Dray *et al.*, 2006), with the first PCNM vectors showing large-scale variation and latter PCNM vectors showing finer scale spatial variation (Borcard *et al.*, 1992). Geographical distance thus represents species patterns emerging from dispersal-limitation and/or unmeasured ecological factors. Variance partitioning analysis (Borcard *et al.*, 1992) was conducted to estimate the relative contribution of component sources of variation (i.e. tree mortality, soil abiotic factors, and geographical distance) in shaping fungal composition at both the community and functional group levels using the *varpart* function in the R package VEGAN (Oksanen *et al.*, 2013). Variables for this analysis were first forward selected based on adjusted coefficients of determination using the FORWARD.SEL function in the R package PACKFOR (Dray *et al.*, 2009). Redundancy analysis (RDA) was performed to interpret the importance of each selected variable in shaping the observed structure in fungal composition.

Results

General characterization of fungal community

A total of 31 542 423 sequences were obtained across all cores. Initial quality filtering reduced this value to 15 988 949 sequences. After rarefaction, 33 550 sequences representing 865 fungal OTUs were assessed for taxonomic affiliation. There were 168 OTUs assigned to ectomycorrhizal fungi, 96 OTUs assigned to saprotrophic fungi, 16 OTUs assigned to arbuscular mycorrhizal fungi and six OTUs assigned to pathogenic fungi. In addition, there were 550 (64%) unidentified fungal OTUs, with most not being assigned to a phylum, and 29 unresolved fungal OTUs in which OTUs were assigned a genus affiliation though their functional grouping was uncertain. Of the 411 fungal OTUs (all identified to phylum) across all sampled soil cores, Basidiomycota was the most dominant phylum and accounted for 24.96% of all OTUs, followed by Ascomycota (19.12%), basal clades of the former Zygomycota (2.42%), and Glomeromycota (1.36%) (Table 1). In Basidiomycota, there were several taxonomic orders representing the majority of OTUs, which included Agaricales, Atheliales, Russulales, and Thelephorales, while in Ascomycota the order Helotiales was the most abundant (Table 1).

Fungal OTU richness

Mean fungal OTU richness across all cores was 85 OTUs (SE = 1.73; $n = 96$). Fungal OTU richness declined across the tree mortality gradient (undisturbed: mean ± SE, 105 ± 5; > 80% attacked: mean ± SE, 83 ± 5), driven by a decline in ectomycorrhizal fungi (undisturbed: mean ± SE, 18 ± 1; > 80% attacked: mean ± SE, 13 ± 1) and saprotrophic fungi (undisturbed: mean ± SE, 26 ± 2; > 80% attacked: mean ± SE, 19 ± 2) (Table 2; Fig. 1). In particular, there was a greater loss of Agaricales (17%), Atheliales (16%), Hypocreales (20%), Mucorales (20%) and Glomerales (14%) across the tree mortality gradient (Table S5). By contrast, Pezizales and Saccharomycetales increased in frequency along the tree mortality gradient. There was a total of 24 indicator OTUs identified across the tree mortality gradient (Table S6). Undisturbed forests harbored a mixture of ectomycorrhizal (e.g. *Cortinarius* spp. and *Suillus* sp.), arbuscular mycorrhizal (e.g. *Glomus* sp.) and dark septate endophyte (e.g. *Phialocephala* sp.) fungi as indicators. By contrast, in

Table 1 Taxonomic distribution of operational taxonomic units (OTUs) making up phyla and orders of soil fungi in lodgepole pine (*Pinus contorta*) forests in Alberta, Canada

Phylum	Order	% OTU
Basidiomycota		24.96
	Agaricales	6.57
	Atheliales	4.01
	Russulales	3.77
	Thelephorales	3.28
	Sebacinales	0.97
	Boletales	0.85
	Other Basidiomycota[1]	1.46
	Unidentified	4.05
Ascomycota		19.12
	Helotiales	6.33
	Pezizales	1.00
	Saccharomycetales	1.00
	Hypocreales	0.73
	Other Ascomycota[2]	3.37
	Unidentified	6.69
Basal lineages		2.42
	Mortierellales	1.94
	Mucorales	0.48
Glomeromycota		1.36
	Glomerales	1.00
	Diversisporales	0.24
	Archaeosporales	0.12
Unidentified		52.14

[1]Includes orders Polyporales, Tremellales, Auriculariales, Cantharellales, and Filobasidiales of the phylum Basidiomycota.
[2]Includes orders Magnaporthales, Chaetothyriales, Eurotiales, Rhytismatales, Capnodiales, Pleosporales, Venturiales, Geoglossales, Peltigerales, Orbiliales, and Archaeorhizomycetales of the phylum Ascomycota.

forests with high tree mortality, substantially fewer ectomycorrhizal fungi were present, while saprotrophic (e.g. Saccharomycetales), pathogenic (e.g. *Verticillium* sp.), and parasitic (e.g. *Tremella* sp.) fungi increased in abundance (Table S6). There were also positive relationships between total fungal OTU richness and soil phenolics and ectomycorrhizal fungal OTU richness and soil phenolics (Table 2). However, total fungal OTU richness as well as ectomycorrhizal and saprotrophic fungal OTU richness did not vary with any other soil abiotic factor (Table 2).

Fungal composition

Overall, fungal community composition correlated with tree mortality ($F_{1,87} = 2.20$; $P = 0.016$), soil nutrients ($F_{1,87} = 2.44$; $P = 0.007$) and geographical distance within plots (PCNM3: $F_{1,87} = 4.91$; $P = 0.038$) and among plots (PCNM1: $F_{1,87} = 71.9$; $P = 0.008$) (Fig. 2a). The community composition of ectomycorrhizal fungi correlated most strongly with tree mortality ($F_{1,87} = 2.22$; $P = 0.023$), soil phenolics ($F_{1,87} = 2.09$; $P = 0.034$) and geographical distance within plots (PCNM3: $F_{1,87} = 4.19$; $P = 0.044$) (Fig. 2b), while the community composition of saprotrophic fungi was correlated with geographical distance among plots (PCNM1: $F_{1,87} = 4.29$; $P = 0.041$) (Fig. 2c). Variance partitioning analyses revealed that the independent effects of tree

Table 2 Models used to test the effects of *Dendroctonus ponderosae*-induced tree mortality, soil nutrients, moisture, and phenolics on the richness of the total soil, ectomycorrhizal and saprotrophic fungi

	Total soil fungi		Ectomycorrhizal fungi		Saprotrophic fungi	
Predictor	$F_{1,84}$	P	$F_{1,84}$	P	$F_{1,84}$	P
Tree mortality	4.93	**0.028**	11.88	**0.009**	11.69	**0.001**
Soil nutrients	0.04	0.830	1.97	0.164	0.21	0.646
Soil moisture	1.79	0.183	2.01	0.159	0.25	0.612
Soil phenolics	5.93	**0.017**	6.73	**0.011**	0.03	0.843

P-values in bold are significant.

mortality (12%), geographical location (15%), and soil abiotic factors (14%) accounted for 41% of the total variation in fungal community composition, while only 9% of the total variation was explained by the shared effect of tree mortality and geographical distance (Fig. 3a). The independent effects of tree mortality (12%) and soil abiotic factors (11%) contributed to most of the variation explained in ectomycorrhizal fungal composition (Fig. 3b), while the independent effect of geographical distance accounted for only a small portion (1%) of the total variation in the composition of saprotrophic fungi (Fig. 3c).

Discussion

Widespread tree mortality caused by mountain pine beetle led to changes in belowground fungal richness and community composition, driven primarily by shifts in ectomycorrhizal fungi. Soil nutrients, soil phenolics and geographical distance also influenced the overall fungal community; however, the relative importance of these factors differed between communities of ectomycorrhizal and saprotrophic fungi. Previous studies have also demonstrated both environmental filtering and geographical distance to be important regulators of fungal communities (Lekberg *et al.*, 2007; Talbot *et al.*, 2014; Taylor *et al.*, 2014). Although the individual components of variation were significant in our study, a majority of the variation in fungal community composition was still unexplained by the factors that were investigated. This was particularly the case for saprotrophic fungi. Factors that may have contributed to the variation in the distribution of these fungi could include environmental conditions not considered here, or possibly stochastic factors such as annual variation in precipitation and temperature (Peay & Bruns, 2014).

In our study, there was an overall decline in belowground fungal community richness and a shift in composition with tree mortality. Ectomycorrhizal fungi depend on their hosts for carbon (Smith & Read, 2008). A direct consequence of tree mortality is presumed to be a severe reduction in carbon flow from these hosts. Our previous work found a similar trend where the richness of ectomycorrhizal fruiting bodies declined and ectomycorrhizal fungal community composition shifted between undisturbed and beetle-killed stands driven by a loss in the dominant tree species, lodgepole pine (Treu *et al.*, 2014). Similarly, a decline in the richness of ectomycorrhizal

fungi was detected from soil 2–3 yr following a European spruce bark beetle (*Ips typographus*) outbreak in a Norway spruce (*Picea abies*) forest in Central Europe (Stursova *et al.*, 2014), and ectomycorrhizal fungal richness and abundance within soils declined by 70–80% following an insect defoliation of mountain birch (*Betula pubescens* ssp. *czerepanovii*) in northern Finland (Saravesi *et al.*, 2015).

Similar to a decline in the richness of ectomycorrhizal fungi, the richness of saprotrophic fungi declined across the gradient of tree mortality. This differs from the findings of our previous work, where saprotrophic sporocarp richness remained invariant across the gradient of tree mortality (Treu *et al.*, 2014). A possible explanation for this disparity is that saprotrophic fungi in this study were sampled from soil, which represented mostly humus-degrading fungi. The decline in both richness (this study) and abundance (Treu *et al.*, 2014) of ectomycorrhizal fungi in soils may have limited the substrate available for decomposition by saprotrophic fungi. In addition to the direct effects of the loss ectomycorrhizal fungi on saprotrophic fungi, our sampling strategy may also have excluded wood-decaying fungi that colonize dying or dead trees, as well as litter-decaying fungi found on the soil surface. There is a pulse of needle deposition following tree mortality in these stands (Cigan *et al.*, 2015); however, dead trees may remain standing for at least a decade post-attack (Lewis & Thompson, 2011). Thus, saprotrophic fungi occurring at the soil surface may not have been limited by substrates to the same extent as fungi occurring belowground.

Composition of the overall fungal community was also influenced, in part, by variation in soil nutrient availability, soil phenolics, and geographical distance. Variation in features of the soil environment, such as those measured in our study, can have a strong influence on soil fungal communities as a consequence of spatiotemporal variability within the soil (Tedersoo *et al.*, 2012; Treseder *et al.*, 2014). Although ectomycorrhizal fungi are directly dependent on their hosts for carbon, they are also sensitive to variation in soil conditions, which

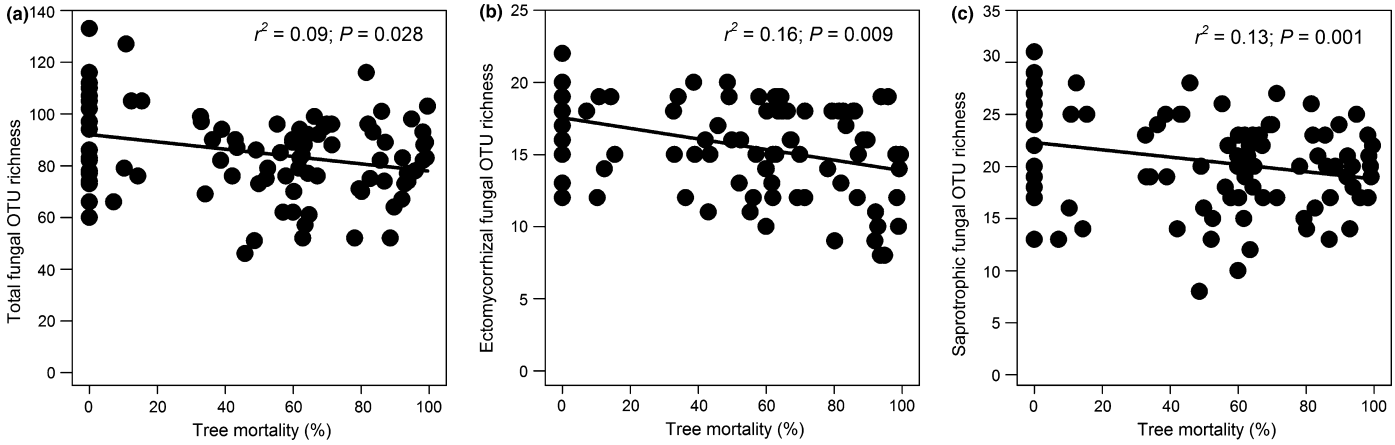

Fig. 1 Response of operational taxonomic unit (OTU) richness of (a) total soil fungi, (b) ectomycorrhizal fungi, and (c) saprotrophic fungi to *Dendroctonus ponderosae*-induced tree mortality.

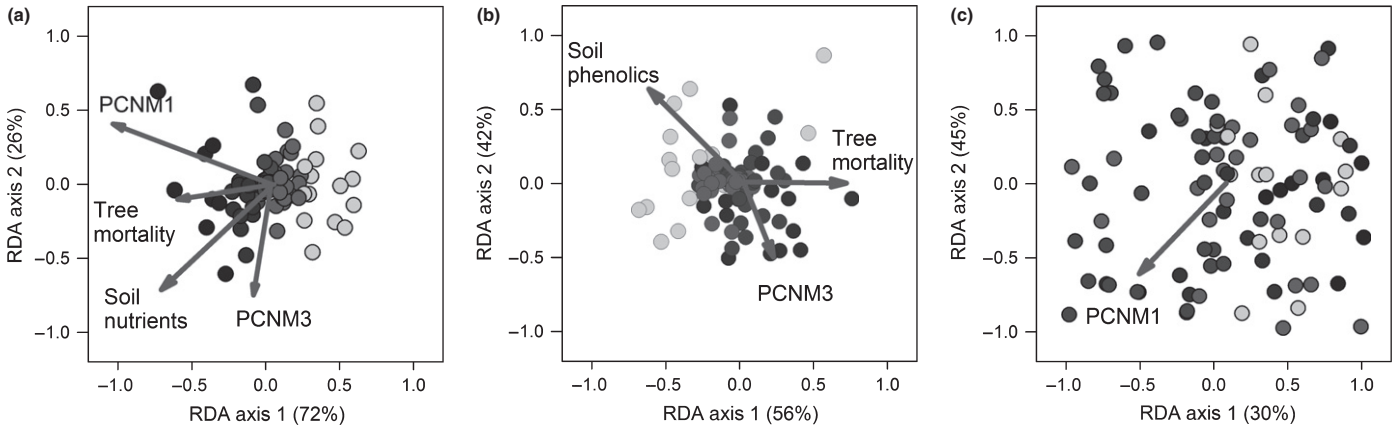

Fig. 2 Redundancy analysis (RDA) ordinations of soil fungal communities: (a) total soil fungal composition, (b) ectomycorrhizal fungal composition, and (c) saprotrophic fungal composition. Total variance explained by each constrained axis is represented. Only significant ($P < 0.05$) predictors are indicated. PCNM1 and PCNM3 predictors represent spatial eigenvectors based on geographical coordinates of sampled soil cores. Principal coordinates of neighbor matrices (PCNM) represent across-site variation while PCNM3 represents within-site variation. Colored dots indicate the extent of tree mortality, with lighter colors representing soil cores taken from undisturbed plots and darker colors representing soil cores taken from beetle-killed sites.

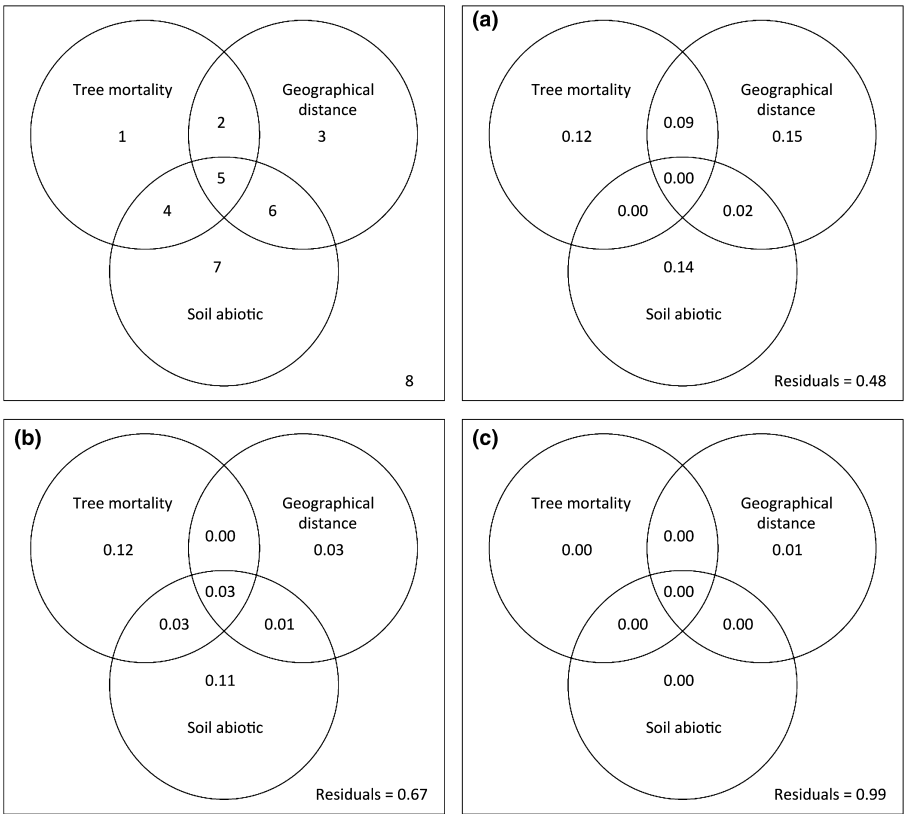

Fig. 3 Variance partitioning of the effects of tree mortality, geographical distance between samples and soil abiotic factors on the community structure of: (a) total soil fungi, (b) ectomycorrhizal fungi, and (c) saprotrophic fungi. Values show the percentage of variation explained by (1) tree mortality, (2) shared contribution of tree mortality and geographical distance, (3) geographical distance, (4) shared contribution of geographical distance and soil abiotic factors, (5) shared contribution of tree mortality, geographical distance, and soil abiotic factors, (6) shared contribution of geographical distance and soil abiotic factors, (7) soil abiotic factors, and (8) the residual unexplained variation.

vary greatly in forested systems (Hättenschwiler & Vitousek, 2000; Ettema & Wardle, 2002; Bahram *et al.*, 2015). Interestingly, the composition of ectomycorrhizal fungi, but not saprotrophic fungi, shifted as soil phenolics decreased with tree mortality (Table S3). Ectomycorrhizal fungi may be sensitive to certain soil phenolic compounds such as benzoic acid and gallic acid, which have been shown to both induce and inhibit ectomycorrhizal growth (Rose *et al.*, 1983; Coté & Thibault, 1988; Kuiters, 1990). Earlier studies have also demonstrated that the community composition of ectomycorrhizal fungi can be altered by the increased production and concentration of phenolic compounds in tree roots (Krupa & Fries, 1971; Napierała-Filipiak *et al.*, 2002). For example, ectomycorrhizal fungi, depending on the stage of colonization, may be inhibited (pre-colonization) or have a neutral response (colonized) to increased concentrations of soil phenolics (Kuiters, 1990). An indirect effect of soil phenolics is their interference with N mineralization (Hättenschwiler & Vitousek, 2000). In our study, the decline of ectomycorrhizal fungi across the tree mortality gradient might partly be explained by the decrease in soil phenolics concentrations and increased soil N availability across this same gradient. Saprotrophic fungi may be less sensitive to the inhibiting properties of soil phenolic compounds, for example, by producing a greater diversity of laccases, which may be involved in the detoxification of phenolic compounds (Kuiters, 1990; Thurston, 1994). However, saprotrophic fungi can be influenced by different soil abiotic factors, such as variation in soil moisture levels and soil nutrient availability (Taylor *et al.*, 2014; Tedersoo *et al.*, 2016), although the influence of resource composition (e.g. dead wood) as a substrate may make them especially vulnerable to changes in forest structure (Hottola *et al.*, 2009). Thus, the unexplained variation in saprotrophic fungal composition during the first few years following mountain pine beetle outbreak might be attributable to a lag effect of resource composition. As it takes a decade or more for >90% of attacked trees to fall following mountain pine beetle outbreak (Mitchell & Preisler, 1998), stochastic processes might govern saprotrophic fungi in soil in the first few years following disturbance, while changes to resources and niche space that potentially occur beyond the timeframe of this study might elicit compositional shifts in saprotrophic fungi.

In conclusion, our results provide novel insight into the underlying mechanisms and outcomes of the effects of widespread tree mortality on soil fungi. Collectively, our results suggest that both environmental and stochastic factors determine the community assembly of soil fungi; however, there were stronger unique than shared effects of tree mortality, soil abiotic factors, and geographical distance on the overall community composition of soil fungi. This suggests that tree mortality, soil chemistry, and geographical distance independently play important roles in structuring the community composition of soil fungi. Though tree death caused by bark beetles triggers a chain of events connected by the plant–soil continuum, these events do not act in unison on soil fungi; rather they appear to act in isolation. Tree species diversity in the boreal forest is relatively low (Perry *et al.*, 2008), and the widespread death of a single common species affects the distribution of hundreds of fungal species directly or indirectly dependent on pine for resources.

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Author contributions

G.J.P., J.K., N.E., J.E.K.C., S.W.S. and J.F.C. planned and designed the research. G.J.P. and P.W.C. conducted field and laboratory work, and G.J.P., J.K. and D.L.T. analyzed data. G.J.P., J.K., D.L.T., P.W.C., N.E., J.E.K.C., S.W.S. and J.F.C. wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Rarefaction curve of observed OTU richness as a function of the number of sequences per sample.

Fig. S2 Sequence distribution of rarified data from next-generation sequencing of fungi occurring in soils.

Table S1 Ion Torrent™ adaptor, primer, and specific multiplex identifier (MID) barcode sequences.

Table S2 Grouping assignments for soil fungi present in soil cores from stands within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta, Canada.

Table S3 Results from Pearson product-moment correlations determining the strength and direction of relationships between tree mortality, soil nutrients, soil moisture, and soil phenolics and macronutrients: calcium, potassium, nitrogen, phosphorus, and sulfur.

Table S4 Summary of soil parameters used in testing the response of the richness and composition of soil fungi to variation in soil moisture, nutrients and phenolics following beetle-induced tree mortality across stands in west-central Alberta, Canada.

Table S5 Operational taxonomic units (OTUs) of fungal phyla and orders gained and lost with tree mortality.

Table S6 List of indicator fungal taxa present in soil cores from undisturbed and severely beetled-killed (>60% *Pinus contorta* killed basal area) stands within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta.

Methods S1 Next-generation sequencing of fungi occurring in soils across a gradient of beetle-induced tree mortality in lodgepole pine (*Pinus contorta*) forests in west-central Alberta, Canada.

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