Spatial patterns, habitat selection and host relationships of *Rhizopogon vesiculosus* and *R. vinicolor* genets in an interior Douglas-fir forest: evidence of vertical niche differentiation

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INTRODUCTION

Ectomycorrhizal (EM) fungi are important in the establishment and growth of northern temperate forests, providing trees with increased access to water and nutrients in exchange for carbohydrates the tree produces by photosynthesis (Leake et al., 2004; Johnson et al., 2006; Taylor, 2006). There is also mounting evidence that the mycelia of some fungal individuals (genets) colonize and potentially link the roots of multiple trees or plants, forming a mycorrhizal network (MN) through which resources may be transferred (Smith & Read 1997; Simard et al. 1997; Leake et al. 2004; Simard & Durall 2004, Lian et al. 2006). However, there is little known about the structure and ecology of ectomycorrhizal fungal communities, or the extent and continuity of individual fungal genets over space and time (Jonsson et al. 1999, Anderson & Cairney 2003, Selosse et al. 2006). This information is needed to relate what is known about the functional aspects of the mycorrhizal symbiosis to broader ecological contexts, such as the role EM fungi play in the establishment, organization and succession of plant communities.

The spatial structure of fungal genets and the genetic structure of populations were historically based on the occurrence of aboveground sporocarps, which are not necessarily associated with the subterranean distribution of fungal mycelia (Gardes & Bruns 1996, Zhou et al. 2001). Advancements in molecular markers and high-throughput DNA identification methods now enable more precise estimates of the spatial patterns of fungi, and with little sampling impact to allow measurements over time (Bonello et al. 1998, Koide et al. 2007). These methods have provided a window into subterranean ecosystems, and researchers are filling the knowledge gaps surrounding the spatial and temporal habits of fungal genets and the niche structures of EM fungal
communities as a whole. In addition, tree roots colonized by EM fungi can also be
identified to species or genotype, allowing studies of fungal specificity regarding the age,
species or genetic makeup of their hosts (Saari et al. 2005; Beiler et al. in press).

*Rhizopogon vinicolor* and *R. vesculosus* (Basidiomycota, *Villosuli*-group sensu
Kretzer et al. 2003a) are among the most frequent and abundant EM colonizers of
interior Douglas-fir trees (Chu-Chou & Grace, 1981; Jones et al., 1997; Twieg et al.
2007), and have been shown to increase seedling growth and resistance towards root
pathogens (Molina et al., 1999). Genets of both *Rhizopogon* species have been shown to
simultaneously colonize seedlings and mature trees, and are thus likely to facilitate the
establishment of new Douglas-fir tree generations through mycorrhizal networks (Beiler
et al., in press). Taxonomy of the *Rhizopogon* (Fries) genus has been in flux since the
seminal work of Smith and Zeller (1966), which placed *R. vesculosus* and *R. vinicolor*
into different sections of subgenus *Rhizopogon* (*Fulviglebae* and *Villosuli* respectively).
Recently these sections have been merged into a new subgenus *Villosuli* sect. *Vinicolores*
by Grubisha et al. (2002) based on nrDNA ITS sequence data and morphological and
ecological similarities. *Villosuli* sect. *Vinicolores* (sensu Grubisha et al) was then further
distinguished by Kretzer et al. (2003a) based on the analysis of nrDNA ITS sequence
data and microsatellite markers. Kretzer et al. (2003b) also investigated the genetic
structure of *R. vesculosus* and *R. vinicolor* populations, whereby the spatial (and
presumably temporal) extent of genets was found to differ between the species.

However, the mechanisms allowing these species to coexist in Douglas-fir forests despite
their similar morphology and ecology remained unknown. Our study sought to address
some of the unanswered ecological questions surrounding these species through fine-
scaled investigations in the field. Our objectives were to describe the frequency of occurrence, vertical and horizontal spatial distributions, substrate preferences, spatial continuity and mycorrhizal networking potential of individual *R. vinicolor* and *R. vesiculosus* genets in an interior Douglas-fir forest.

**MATERIALS AND METHODS**

**Site characteristics**

This study took place in a mixed-aged interior Douglas-fir forest in the dry, cool interior Douglas-fir biogeoclimatic subzone (IDFdk2, Thompson variant) near Kamloops, British Columbia, Canada (51° 51.1-51.6’ N latitude, 120° 31.0-31.6’ W longitude). Genets of *R. vesiculosus* and *R. vinicolor* were investigated in six plots, including three plots having either dry or moist soil conditions relative to the IDFdk. Soil moisture regimes were distinguished based on mesoslope position (upper and lower slopes) and indicative soil and vegetation characteristics as per Lloyd *et al* (1991). In short, dry plots (“Ex1-3”) were located on upper slope positions with subxeric soils and an understory dominated by pinegrass (*Calamagrostis rubescens*, Buckley) with interspersed common juniper (*Juniperus communis*, Thunb.). Moist plots (“Ex4-6”) were located on lower slope positions with permesic soils and a more diverse assemblage of understory plants, including a greater percent cover of red-stemmed feathermoss (*Pleurozium schreberi*, Brid.), soopolallie (*Shepherdia Canadensis*, Nutt.), twinflower (*Linnaea borealis*) and prince’s pine (*Chimaphila umbellate*) among other species. Though we compared sites with moist and dry soils, these forests are generally water stressed throughout much of the growing season, with approximately 44 cm of annual precipitation falling primarily as
snow during winter months (Lloyd et al. 1990; Hope et al. 1991; Joy & Maclauchlan 2000). Mean maximum and minimum temperatures are 21.0°C and -4.2°C (mean annual = 3.4°C), with an average growing season of 166 days per year (Environment Canada; Canadian climate normals 1971-2000; http://climate.weatheroffice.ec.gc.ca). Elevation of the study sites ranged from 996-1,155 m above sea level and all had a gentle slope (0-20%) and southwestern aspect. The forest floor was predominantly Mull Moder with depths ranging from 1-11 cm. Douglas-fir lateral roots were observed up to 33 cm depth, with most fine roots in the upper 15 cm. Mineral soils were silty clay loam Luvisols (Canadian System of Soil Classification, 1998).

**Sampling methods**

We used three different sampling schemes, which combined contiguous and systematic sampling, to augment the resolution and scale (sampling “grain” and “extent”) at which individual genets were measured (Fortin & Dale 2005). First, at all six sites contiguous 20 cm³ soil blocks were excavated in transects spanning a minimum of 2 m length x 20 cm width x 20 cm depth in 2 directions (≥10 adjacent 20 cm³ soil blocks in 2 directions; sites Ex1-6). Second, in one of the six plots (site Ex4) a contiguous 1 x 2 m area, surrounded by a 1 m buffer sampled as a lattice with a 20 x 20 cm spatial lag between samples (checkerboard pattern) was sampled in addition to the initial contiguous transects. And third, one of the six plots (site Ex6) was sampled to a 2 x 1.6 m extent in the checkerboard lattice pattern surrounding the contiguous transects. In each 20 cm³ soil block, the L horizon of the forest floor was sampled separately, followed by a pooled
sample of the F and H forest floor layers in combination with coarse woody debris, and finally a pooled sample of A and B mineral horizons.

The frequency of *R. vesiculosus* and *R. vinicolor* was measured relative to the number of 20 cm$^3$ soil blocks sampled (contiguous transects only) by site, moisture regime, and overall. The brown mycelial growth of *Rhizopogon* genets was readily identifiable in the field, and was classified into five density classes including absent, scarce, diffuse, patchily dense, and dense. These density classes were based on the number of EM root tips present, mycelial growth type (e.g. diffuse rhizomorphs versus dense mats of hyphae), and the percent of the sampling area occupied. Any samples that could not be identified following molecular processing were presumed to be dead or not the target species. Mycelial density was classified separately in each soil layer, and each layer was further subdivided into four 10 x 10 cm quadrants to estimate the percent area occupied by fungal vegetative growth. Substrate type (rock surface, coarse woody debris, L, H & O organic layers, or A & B mineral layers) and depth (cm) was recorded for each sample location. Two or more samples of *Rhizopogon* spp. mycorrhizas, hyphae, or rhizomorphs were collected from each soil block and strata (when available), placed in 2 ml polypropylene tubes, and frozen at -20°C for molecular analysis.

**Molecular analysis**

*Rhizopogon*-like strands of hyphae or rhizomorphs and individual root tips within tuberculate ectomycorrhizas were isolated from field samples under a stereomicroscope at 20x magnification. A random subsample of 0.1 g of this material underwent molecular processing (including DNA extraction, PCR amplification of microsatellite loci and
fragment analysis) as described in Beiler et al. (in press). Douglas-fir tree DNA isolated from EM samples was genotyped at microsatellite loci PmOSU_1C3, PmOSU_1F9 and PmOSU_2D4 using primers developed by Slavov et al. (2004). We used primers developed by Kretzer et al. (2003b) to genotype R. vesiculosus at the microsatellite loci Rv02, Rv15, Rv46, Rve1.21, Rv1.34, Rve2.10, Rve2.44, Rve2.77 and Rve3.21; and R. vinicolor at loci Rv02, Rv15, Rv46, Rv53, Rv1.34, Rve2.10, Rve2.14, Rve2.77, and Rve3.21. Two or more samples were considered to represent an individual if they had matching alleles at all microsatellite loci analyzed. The probability of identity of Douglas-fir and Rhizopogon spp. genotypes was estimated with the inclusion of samples collected from a larger-scaled study in the vicinity of our study plots and reported in Beiler et al. (in press).

**Data analysis and statistics**

The frequency of occurrence, mean mycelia depth of genets, and mean depth of EM samples was compared between R. vesiculosus and R. vinicolor and between soil moisture regimes using Wilcoxon Rank Sums tests. Habitat preferences of R. vesiculosus and R. vinicolor among available substrates were assessed using Manley’s alpha habitat selectivity index as described in Krebs (1989). The mean depth of the forest floor (L, H & O organic layers) was compared between soil blocks occupied by R. vesiculosus versus those of R. vinicolor, and between dry and moist soils, using two-sample t-tests. The mean number of trees colonized by genets was also compared between Rhizopogon spp. and between soil moisture regimes using two-sample t-tests. Mycelial density was
compared between Rhizopogon spp. and between soil moisture regimes independently for each species using Chi-squared likelihood ratio tests.

RESULTS & DISCUSSION

Rhizopogon spp. frequency of occurrence

A total of eight R. vesiculosus and seven R. vinicolor genets were encountered across all six contiguously sampled transect plots. Each plot had only one genet from either Rhizopogon species, with the exception of site Ex6 which had 2 genets from each species. R. vesiculosus was encountered in all six plots, while R. vinicolor was found in four of six plots (2/3 dry and 2/3 moist sites). Overall, R. vesiculosus occurred in 45.8 ± 30.4% of the 20 cm³ soil blocks sampled and R. vinicolor occurred in 25.0 ± 24.1%. There were no significant differences in the frequency of occurrence between Rhizopogon spp. or between plots having moist versus dry soil conditions.

That R. vesiculosus was encountered in every plot surveyed, and with such a high frequency of occurrence among sampling blocks within plots, emphasizes the dominance of this species as reported from earlier studies of EM communities in Douglas-fir forests (Twieg et al. 2007; Roth & Berch 1992; Molina et al. 1999). In effect, R. vesiculosus occupied nearly 50% of the study area based on a 20 cm³ sampling resolution; twice the frequency observed for R. vinicolor in the study. These differences are consistent with the larger genet sizes previously reported for R. vesiculosus compared to R. vinicolor, since similar numbers of genets are often reported per unit area for these species (Kretzer et al. 2003b, Beiler et al., in press). Our results suggest R. vesiculosus and R. vinicolor
are not only important as Douglas-fir symbionts, but also for ecosystem services such as nutrient cycling, carbon sequestration and the formation of fertile soils.

**R. vesiculosus and R. vinicolor ecology**

**Spatial distributions and segregation:** Based on the visible presence of *Rhizopogon*-like mycelial growth, *R. vesiculosus* genets were encountered significantly deeper than *R. vinicolor* genets when averaged for each sampling block and then across genets ($n_1 = 8$, 2.5-27.0 cm, mean = 11.3 ± 4.3 cm; $n_2 = 7$, 2.0-17.0 cm, mean = 6.1 ± 1.9 cm; $Z = -6.36$, $p < 0.001$). The depth at which EM samples were collected was also significantly deeper for *R. vesiculosus* compared to *R. vinicolor* ($n_1 = 148$, 2.5-27.0 cm; mean = 11.5 ± 6.6 cm; $n_2 = 68$, 2.0-17.0 cm; mean = 7.2 ± 4.3 cm; $Z = -4.52$, $p < 0.001$). These results corroborate the trend we observed in a previous study based on the dispersed sampling of *R. vesiculosus* and *R. vinicolor* at larger spatial scales (Beiler *et al.*, in press). Though we did not observe any spatial overlap between different genets of the same species, spatial overlap did occur between the two *Rhizopogon* species. We found that where genets of *R. vesiculosus* and *R. vinicolor* overlap in horizontal space, they partition vertically in the soil horizons. Additionally, *R. vinicolor* appears to inhabit primarily the uppermost layers of the forest floor and organic soil horizons, while *R. vesiculosus* has a broader range of depth which includes mineral soil horizons.

The vertical partitioning exhibited by these closely related species may explain their co-occurrence within Douglas-fir forests despite their shared morphology and ecology. Niche differentiation between different ECM species has been documented in the field (Goodman & Trofymow, 1998; Tedersoo *et al.*, 2003; Baier *et al.*, 2006; Genney
et al., 2006), and could underlie functional diversity in ectomycorrhizal fungal

220 communities (Dickie et al., 2002; Rosling et al., 2003; Anderson & Cairney, 2007). For example, the greater depth range of *R. vesiculosus* may provide greater access to water and nutrients deeper in the soil during dry periods, thereby promoting the water stress tolerance of its hosts. However, given the considerable overlap between the fundamental niches of *R. vesiculosus* and *R. vinicolor*, competitive interactions may better explain the coexistence of these species through a shared preference model of niche differentiation.

225 In an experimental microcosm, Kennedy and Bruns (2005) reported the competitive dominance of *Rhizopogon occidentalis* versus *R. salebrosus* when given first priority colonizing *Pinus muricata* seedlings. The competitive interactions between *R. vesiculosus* and *R. vinicolor*, and the relationships between their depth of occurrence and genet size, growth rate and longevity, should be the subject of future studies.

Substrate preferences: Based on Manley’s habitat selection index, both *Rhizopogon* species in this study shared similar habitat preferences with respect to aboveground vegetation cover and belowground substrate types (Table 1). Of the available types of ground cover, both *R. vesiculosus* and *R. vinicolor* selected for coarse woody debris and rock cover (rocks typically 20 cm diameter or larger). Previous reports also associated *R. vinicolor* with coarse woody debris, though the relationship was never empirically tested. Because Manley’s alpha is based on the ratio of habitat use : availability, this suggests that a higher proportion of these habitats were occupied by *Rhizopogon* spp. than would be expected by chance. This is significant because both rock cover and coarse woody debris are associated with soil moisture retention. Given the moisture deficits of these
forests, it would be expected there would be fierce competition for these microhabitats among the more than one hundred EM species that inhabit interior Douglas-fir forests. The ability of *R. vesiculosus* and *R. vinicolor* to pervade these habitats provides further evidence of the key role these species play in the ecosystem. And while *R. vesiculosus* and *R. vinicolor* were most prevalent beneath coarse woody debris and rocks, they were not absent beneath other cover types. In fact, *Rhizopogon* spp. EM root tips were encountered below every cover type investigated from bare ground to common juniper shrubs.

Among belowground substrates, both species again favored coarse woody debris, as well as the interface of organic and mineral soil layers (H & A horizons), while *R. vinicolor* also clearly selected for the F & H horizons of the forest floor. These results are not surprising, given that the majority of Douglas-fir fine roots also occurred within these soil horizons, where the highest rates of gas exchange and nutrient accumulation occurs. The results do, however, reiterate the fundamental niche overlap between these species. Interestingly, we also found that the depth of the forest floor (including L, F, & H horizons) was significantly deeper in sampling blocks where *R. vinicolor* was encountered (n = 59, mean = 6.51 ± 2.36) verses those with *R. vesiculosus* (n = 103, mean = 5.06 ± 2.62) (t = 6.31, p < 0.001). Thus we may speculate that *R. vinicolor* is particularly adapted to the upper layers of soil and outcompetes *R. vesiculosus* in this habitat. And conversely, though *R. vesiculosus* is outcompeted in the upper reaches (in vertical space) of its fundamental niche, the species is able to persist within its realized niche by retreating into deeper soils. Though the competitive interactions between these species remain unresolved, similar patterns of niche divergence among functional guilds
have been described for many different communities of organisms including bacteria, fungi, diatoms, insects, fish, plants and mammals.

**Mycorrhizal networking**

*Rhizopogon spp. genets as links between tree roots:* We found that every fungal genet (except one represented by only one sample) colonized more than one tree ($n = 9$, range = 2-9, mean $= 4.33 \pm 2.63$). Genets of *R. vesiculosus* ($n = 5$, 3-9; mean $= 5.80 \pm 2.59$) colonized significantly more trees than those of *R. vinicolor* ($n = 4$, 2-3; mean $= 2.50 \pm 0.58$) among soil blocks in the six contiguous transects ($t = -2.77; p = 0.04$) (Figure 3). This difference was even more pronounced with the inclusion of samples collected within the extended sampling areas of sites Ex4 & Ex6 ($n_1 = 5$, mean $= 7.40 \pm 3.05$; $n_2 = 5$, mean $= 3.20 \pm 1.30$; $t = -2.83; p = 0.03$). No significant difference in the number of trees colonized by genets was detected between dry and moist soils for either species.

Earlier studies have shown that *Rhizopogon* genets associate with numerous host trees, and that *R. vesiculosus* associates with and potentially links more trees through mycorrhizal networks than *R. vinicolor*, but the continuity of fungal mycelia spanning roots from differing trees had not been demonstrated (Beiler et al., in press). In this study, nearly ten percent of the 20 cm$^3$ blocks sampled contained roots from multiple trees colonized by a single fungal genet, such that trees were connected within very close proximity of each other. We also encountered individual trees colonized by the same fungal genet repeatedly (i.e. in network terminology, these represent loops) across the areas sampled, suggesting that even if the continuity of a mycorrhizal network becomes
disrupted, the opportunity exists for the mycelia of the linking genet to persist and re-
bridge those gaps through anastamosis.

290 **Genet continuity:** In site Ex4 where soil blocks were sampled contiguously in two
dimensions, linear continuity was observed between 60% of neighboring soil blocks for
*R. vesiculosus* but only 30% of neighboring blocks for *R. vinicolor* (Figure 4). Thus
while *R. vesiculosus* was highly continuous across space, the occurrence of *R. vinicolor*
appeared more sporadic and genotypes of this species may represent a collection of
fractioned clonal ramets rather than continuous genets. This suggests that *R. vesiculosus*
genets are not only larger and colonize more trees compared to *R. vinicolor* genets, but
are also more likely to link colonized trees across the span of those genets. A similar
pattern was observed among the contiguously sampled transect plots, though the
likelihood of observing continuity in these plots was restricted by their one-
dimensionality (Figure 3). Because the scale of our sampling did not capture entire
genets or the patch structure of genets across the landscape, we were unable to quantify
the upper limits of spatial continuity for either *Rhizopogon* spp. However, our findings
that both *R. vesiculosus* and *R. vinicolor* genets exhibit linear continuity at least at small
scales, together with evidence of multiple trees being colonized within close proximity
(20 cm³), supports the assumption that individual genets of both species may link the
roots of multiple trees through uninterrupted pathways. And in the case of *R.
vesiculosus*, the “long-distance exploration-type” rhizomorphs formed by this species
could be particularly effective in the translocation of nutrients and water to host trees
over long distances (Duddridge *et al.* 1980; Read and Boyd 1986; Agerer 2001).
Mycelial density of *R. vesiculosus* and *R. vinicolor* genets

The density of mycelia (including EM root tips, hyphae and rhizomorphs) among 20 cm$^3$ sampling blocks was greater for *R. vesiculosus* than for *R. vinicolor* ($n_1 = 150$, $n_2 = 78$; $X^2 = 9.89$, df = 3, $P < 0.02$) (Figure 5). In addition, the mean mycelial density of genets was greater among sites with moist soils compared to those with dry soils for both *R. vesiculosus* ($n_{dry} = 52$, $n_{moist} = 98$, $X^2 = 15.13$, $p < 0.01$) and *R. vinicolor* ($n_{dry} = 20$, $n_{moist} = 58$, $X^2 = 12.36$, $p < 0.01$). Spatial autocorrelation of mycelial density across horizontal space was not detected for either species (data not shown) and there was visible variability among adjacent soil blocks. In this sense, the mycelia of genets exhibiting linear continuity were characterized by dispersed patches of dense growth linked through scarce or diffuse hyphae and rhizomorphs (Figures 3 & 5).

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microsatellite markers allow roots and ectomycorrhizas to be linked to individual


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**Table 1.** Preferred types of ground cover and belowground substrates of *R. vesiculosus* and *R. vinicolor* genets based on Manley’s habitat selectivity index (Manley’s alpha). Plus signs indicate a habitat use : availability ratio higher than the significance threshold, Manley’s alpha, while negative values represent ratios below alpha.

**Figure 1.** Top-down view of the spatial distribution and mean depth of occurrence (cm) of the vegetative growth (hyphae, rhizomorphs and EM root tips) of *Rhizopogon vesiculosus* (a) and *R. vinicolor* (b) within a 1 x 2 m plot (site “Ex4”) sampled in contiguous 20 cm³ increments.

**Figure 2.** Mean depth of occurrence (cm) of *Rhizopogon* spp. mycelia (hyphae, rhizomorphs and EM root tips), averaged across 20 cm³ sample blocks and genets. Boxplots show the range (wiskers), median (middle line) and 1st and 3rd quartiles of genet depth means.

**Figure 3.** Blocks illustrating the continuity of the occurrence and density of *R. vesiculosus* and *R. vinicolor* genets within six contiguously sampled 20 cm x 2 m transects, with three sites each in dry or moist soils relative to the dry, cool interior Douglas-fir biogeoclimatic zone, and with the upper and lower 10 cm of soil sampled separately.

**Figure 4.** Boxplots showing the mean number of trees colonized by individual fungal genets, compared between the EM species *Rhizopogon vesiculosus* and *R. vinicolor*. Lines represent the range of genet means (wiskers), the median (center lines), and the first and third quartiles (top and bottom of boxes).

**Figure 5.** Top-down view of the distribution and density of belowground mycelia (hyphae, rhizomorphs, and EM root tips) identified as *R. vesiculosus* (a) and *R. vinicolor* (b) within the same 1 x 2 m plot (site “Ex4”) sampled in contiguous 20 cm³ increments.
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Plus signs indicate a habitat use : availability ratio higher than the significance threshold, Manley’s alpha, while negative values represent ratios below alpha.

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<th>Vegetation cover type</th>
<th>\textit{R. vesiculosus}</th>
<th>\textit{R. vinicolor}</th>
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<tr>
<td>coarse woody debris</td>
<td>+</td>
<td>+</td>
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<tr>
<td>duff</td>
<td>-</td>
<td>+</td>
</tr>
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<td>rock</td>
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<td>Douglas-fir</td>
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<th>\textit{R. vinicolor}</th>
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<td>organic (F + H)</td>
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<td>against rock</td>
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<td>-</td>
</tr>
<tr>
<td>coarse woody debris</td>
<td>+</td>
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Figure 1. Top-down view of the spatial distribution and mean depth of occurrence (cm) of the vegetative growth (hyphae, rhizomorphs and EM root tips) of *Rhizopogon vesiculosus* (a) and *R. vinicolor* (b) within the same 1 x 2 m plot (site “Ex4”) sampled in contiguous 20 cm³ increments.
Figure 2. Mean depth of occurrence (cm) of *Rhizopogon* spp. mycelia (hyphae, rhizomorphs and EM root tips), averaged across 20 cm³ sample blocks and genets. Boxplots show the range (whiskers), median (middle line) and 1ˢᵗ and 3ʳᵈ quartiles of genet depth means.
Figure 3. Blocks illustrating the continuity of the occurrence and density of *R. vesiculosus* and *R. vinicolor* genets within six contiguously sampled 20 cm x 2 m transects, with three sites each in dry or moist soils relative to the dry, cool interior Douglas-fir biogeoclimatic zone, and with the upper and lower 10 cm of soil sampled separately. Blocks with *R. vesiculosus* present are colored in shades of blue and *R. vinicolor* with shades of red, with the darkness of colors increasing with density; blocks with *Rhizopogon*-like EM tubercules present that were dead and/or could not be identified with molecular analysis are shaded in gray. Density of *Rhizopogon* spp. mycelia was rated as absent (no visible *Rhizopogon*-like mycelia), scarce (≤ 1 EM root tip and sparse hyphae & rhizomorphs covering ≤ ½ of sample block), diffuse (≤ 10 EM root tips and sparse hyphae or rhizomorphs covering > ½ of sample block), patchily dense (≥ 10 EM root tips or with dense hyphal mats covering ≤ ½ of sample block), or dense (≥ 10 EM root tips or with dense hyphal mats covering > ½ of sample block).
Figure 4. Boxplots showing the mean number of trees colonized by individual fungal genets, compared between the EM species *Rhizopogon vesiculosus* and *R. vinicolor*. Lines represent the range of genet means (wiskers), the median (center lines), and the first and third quartiles (top and bottom of boxes).
Figure 5. Top-down view of the distribution and density of belowground mycelia (hyphae, rhizomorphs, and EM root tips) identified as *R. vesiculosus* (a) and *R. vinicolor* (b) within the same 1 x 2 m plot (site “Ex4”) sampled in contiguous 20 cm³ increments.