Saprotrophic and ectomycorrhizal fungal sporocarp stoichiometry (C : N : P) across temperate rainforests as evidence of shared nutrient constraints among symbionts

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Saprotrophic and ectomycorrhizal fungal sporocarp stoichiometry (C:N:P) across temperate rainforests as evidence of shared nutrient constraints among symbionts

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Summary

- Quantifying nutritional dynamics of free-living saprotrophs and symbiotic ectomycorrhizal fungi (EMF) in the field is challenging, but the stoichiometry of fruiting bodies (sporocarps) may be an effective methodology for this purpose.
- Carbon (C), nitrogen (N), and phosphorus (P) concentrations of soils, foliage and 146 sporocarp collections were analyzed from 14 *Pseudotsuga menziesii* var. *menziesii* stands across a podzolization gradient on Vancouver Island (Canada).
- N and P concentrations were considerably higher in saprotrophic fungi. Fungal N% increased with soil N content at a greater rate for saprotrophs than EMF, while fungal P% of saprotrophs was more constrained. Fungal N:P was more responsive to soil N:P for EMF (homeostatic regulation coefficient 'H' =2.9) than saprotrophs (H= 5.9), while N:P of EMF and host tree foliage scaled almost identically.
- Results underscore the role of EMF as nutrient conduits, supporting host trees, whereas saprotrophs maintain a greater degree of nutritional homeostasis. Site nutrient constraints were shared in equal measure between EMF and host trees, particularly for P, suggesting neither partner benefits from enhanced nutrition at the expense of the other. Sporocarp stoichiometry provides new insights into mycorrhizal relationships and illustrates pervasive P deficiencies across temperate rainforests of the Pacific Northwest.

Keywords: mycorrhiza, mutualism, podzolization, phosphorus deficiency, ecosystem retrogression, holobiont

Introduction

Free-living saprotrophic and symbiotic ectomycorrhizal fungi (EMF) are distinct, 'hyperdiverse', cohabitating guilds of forests ecosystems (Taylor *et al*., 2014). The guilds share a common ancestry, with the symbiotic habit of ectomycorrhiza considered to have evolved independently among multiple lineages of free-living fungi (Tedersoo & Smith, 2013). Both guilds contribute to soil organic matter decomposition and nutrient mobilization, although the full extent of cellulolytic and ligninolytic activities among EMF is of some debate (Lindahl & Tunlid,2015; Martin *et al*., 2016; Uroz *et al*., 2016). Nevertheless, these fungi share a number of traits that enable the effective exploitation of limited, spatially heterogeneous soil resources, particularly nitrogen (N) and phosphorus (P) (Finlay, 2008). These traits include large increases in absorptive surface area via filamentous hyphae, the production of extracellular hydrolytic and oxidative enzymes, the exudation of low molecular weight organic acids, low and high affinity membrane transporters, and direct absorption of organic N and (possibly) P forms (Treseder & Lennon, 2015; Hodge, 2017). Studies of forest ecology have increasingly drawn attention to the important competitive interactions among saprotrophs and EMF through their influence on processes such as nutrient turnover and soil carbon (C) sequestration (Fernandez & Kennedy, 2016). For this reason our understanding of forest ecosystems would likely improve with more detailed studies of all the interacting soil fungi in an ecosystem, rather than studying either guild in isolation.

Nutrient stoichiometry (element ratios of C:N:P) of biota has been widely applied in marine and terrestrial ecology to assess nutrient availability, cycling and ultimate limitations to ecosystem productivity (Sterner & Elser, 2002). For microbial communities in particular, ecological stoichiometry has the potential to elucidate nutritional constraints across ecosystems and adaptations to environmental heterogeneity (Mooshammer *et al*., 2014; Zeichmeister-Boltenstern *et al*., 2015). Reviews of soil microbe stoichiometry have emphasized the relative homeostasis of microbial biomass, but have also highlighted significant variation among biomes and habitats (Cleveland & Liptzin, 2007; Hartman & Richardson, 2013; Xu *et al*., 2013). In coniferous forests, some of the variation in microbial stoichiometry could reflect the fundamental differences in nutrient uptake and retention between free-living and symbiotic fungal guilds. Saprotrophic fungi obtain and retain nutrients for their own needs in growth and reproduction, utilizing soil C for energy, whereas symbiotic fungi transfer a portion of their nutrients to the host tree in exchange for C-rich photosynthate (Mayor *et al*., 2009). We suggest this distinction underlies the nutritional patterns reflected in studies of terrestrial (soil and humus substrates) forest fungi, where EMF generally have lower concentrations of N compared to saprotrophs (Vogt *et al*., 1981; Gebauer & Taylor, 1999; Trudell & Edmonds, 2004; Trocha *et al*., 2016).

Basic distinctions and patterns in fungal stoichiometry such as these have only recently been characterized, and there is a need for further testing of nutrient relationships by fungal guild over a range of abiotic conditions (Zhang & Elser, 2017). Temperate coastal forests of the Pacific Northwest (United States and Canada) encompass a wide amplitude in soil N regimes through a combination of contrasting parent materials, topography and climate (Carpenter *et al*., 2014). In addition, the more humid, coniferous rainforests of the Pacific west coast have undergone accelerated podzolization (Sanborn *et al*., 2011), resulting in sharp reductions in soil inorganic P availability due to strong sorption with reactive (iron and aluminum oxides) soil components (Preston & Trofymow, 2000). It has been noted that these temperate rainforests may therefore be in a phase of ecosystem retrogression, where P deficiencies are more fundamentally limiting to productivity than N (Kranabetter *et al*., 2005). As decomposers, fungal communities directly influence resource availability through the balance of nutrient retention and release (Zechmeister-Boltenstern *et al*., 2015), and consequently, fungal stoichiometry could mirror these underlying patterns in contrasting soil N:P, perhaps even more clearly than vegetation (Cleveland & Lipzin, 2007). There may, however, be a distinction in stoichiometry between free-living and symbiotic fungi. We hypothesize the nutrient status of EMF to be strongly constrained (near constant C:N and C:P) because in a mutualism, any 'excess' N or P not essential to fungal metabolism would be passed on to the host to maximize fitness (*sensu* optimal foraging theory; Johnson, 2009). Saprotrophic fungi are perhaps more capable of non-homeostatic behaviour because of physiological adjustments that allow for the storage of available nutrients (Mooshammer *et al*., 2014).

Much of the research into microbial stoichiometry has relied upon bulk soil fumigations which do not allow for testing of nutritional trends by functional guild or species. The homogenization of disparate soil microflora likely contributes to the variability in microbial stoichiometry reported across biomes (Hartman & Richardson, 2013; Xu *et al.*, 2013), especially as EMF in forest soils can comprise a substantial proportion of total microbial biomass (Anderson & Cairney, 2007). Fruiting bodies (sporocarps) may facilitate stoichiometry studies as they provide a very efficient and specific sampling regime of fungal tissue for a large number of species (Vogt & Edmonds, 1980). In this study we present sporocarp nutrient concentrations and element ratios of both saprotrophic and EMF species over an edaphic gradient of N and P availability caused by an orographic rainshadow along southern Vancouver Island (British Columbia, Canada). Foliar nutrition of the primary producer (and EMF plant host) in these ecosystems, coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco), was included for comparison. Our hypotheses, as alluded to above, were that EMF as symbiotic organisms will have: 1) lower concentrations of N and P than saprotrophic fungi; 2) stronger homeostasis in C:N and C:P ratios

with increasing soil nutrient availability than saprotrophs; and 3) more stable N:P ratios over the edaphic gradient than either saprotrophic fungi or the tree host. The results of our study will establish whether nutrient content and element ratios can define the distinctions between free-living and symbiotic fungal guilds, and indicate how key ecosystem processes, such as podzolization (Turner *et al.*, 2012) and retrogression (Peltzer *et al*., 2010), are reflected in fungal stoichiometry.

Materials and Methods

Site descriptions

A total of 14 sites were selected across southern Vancouver Island (extending approx. 100 km) to encompass edaphic gradients in: 1) P availability via the extent of soil podzolization between dry maritime (referred to as 'upland Brunisol') and wet maritime ('upland Podzol') mesotrophic forests; and 2) N availability between upland sites and nutrient-rich, moist 'lowland Podzol' forests (Table 1). All study sites were low elevation (< 400 m), second-growth coastal Douglas-fir plantations, between 40-60 years in age, which were established as part of long-term silvicultural studies of the B.C. Forest Service. Plot size was set at 25 × 25 m and we established 4-5 replicates of each site type. Two sites (Branch 167 and 247) were large research installations over an area of complex topography that enabled us to sample both an upland and lowland Podzol plot (approx. 400 m apart at both sites).

Upland Brunisol (equivalent to Inceptisol or Cambisol; Soil Classification Working Group 1998) plots were located in the Coastal Western Hemlock very dry maritime subzone (CWHxm; Green and Klinka 1994) of eastern Vancouver Island (between Victoria and Duncan, B.C.) along mid-slope positions with modest slope grades (30-60%). These glacial morainal soils are typically well drained with sandy loam textures, moderate stone content, and thin (approx. 1 cm) forest floors. Sites are characterized by a high understory cover of shrubs, including *Gaultheria shallon*, *Mahonia nervosa*, *Vaccinum parvifolium*, and mosses *Hylocomium splendens* and *Kindbergia oregana*. Mean annual precipitation of these sites is relatively low for the west coast (averaging 1370 mm; Table 1) due to their location on the leeward side of an orographic rainshadow created by the Vancouver Island and Olympic mountains.

Upland Podzol (= Spodosol) plots were located in the Coastal Western Hemlock very wet maritime subzone (CWHvm; Green and Klinka 1994) of western Vancouver Island (between Port Renfrew and Bamfield, B.C.), where mean annual precipitation averages almost 3400 mm (Table 1). These sites are found on modest to steep slopes (40-100%), with primarily glacial morainal soils (one colluvial site at WC1000) of sandy loam to loam texture, moderate stone content and varying forest floor depth (1-10 cm, average of 5.5 cm). Sites are characterized by a shrub layer of *Vaccinum parvifolium*, *Vaccinium alaskaense* and *Gaultheria shallon*, and a well-developed moss layer dominated

by *Hylocomium splendens* and *Rhytidiadelphus loreus*. Herbs include minor amounts of *Blechnum spicant* and *Rubus pedatus*. The colluvial site at WC1000 had a more extensive cover of *Polystichum munitum* and *Tiarella trifoliata*.

Lowland Podzol plots were also located in the very wet maritime subzone (CWHvm) of western Vancouver Island and were characterized by seasonal water table fluctuations, either due to seepage (high bench floodplains and lower toeslopes) or impeded drainage caused by duric horizons in the lower profile. Soils were glacial morainal or glacial fluvial in origin, with sandy loam to loam textures and varying forest floor depth (1-8 cm, average of 4.2 cm). Understories were lush and comprised of shrubs, particularly *Rubus spectabilis*, and many herb species, including *Polystichum munitum*, *Dryopteris expansa*, *Tiarella trifoliata*, *Maianthemum dilatatum*, and *Athyrium filix-femina*. Representative images of the three forest ecosystems with soil profiles are presented in Supporting Information Figure S1.

Soil, foliar, sporocarp sampling and analysis

In May of 2017 we sampled the upper soil profile for chemical properties on 11 plots (Kapoor, Sooke and Niagara were sampled in the same manner with the same field personnel in 2015). Forest floors were cut and removed over a 10 cm diameter area, while mineral soils were retrieved to a 20 cm depth with a stony soil auger. Subsamples from 12 random microsites were combined into 3 forest floor and 3 mineral soil samples per plot (an exception were upland Brunisol plots, where forest floors were so thin that one bulked sample per plot was taken). Soils were air-dried, ground and sieved to 2 mm for chemical analysis. Foliar samples of all 14 plots were taken at the end of the growing season (mid-November 2017) by searching each plot for fresh branches of Douglas-fir that had broken off during recent storms. We obtained needles from current year foliage off 12 separate branches and bulked these into 3 samples per plot. Foliar samples were oven-dried at 60°C for 24 hours and then ground for nutrient analysis.

Each plot was searched twice, about 3 weeks apart, for fleshy, terrestrial saprotrophic and EMF sporocarps in the fall of 2017 (mid-October to mid-November). Fungal species fruiting on coarse woody debris were avoided as this very nutrient-poor substrate would not correspond well with the underlying edaphic gradient in N and P availability (Gebauer & Taylor, 1999). We selected sporocarps that were fresh and free from insect damage; almost all collections had collembola (spring tails) scattered on the cap and stipe but these fauna were easily dislodged and removed upon drying. We strove to obtain composite samples for each fungal species, typically 3-10 individual sporocarps, from over the entire plot area. For some of the smaller saprotrophic genera, such as *Mycena*, we collected all the individuals we could find to obtain enough tissue for analysis. Sporocarps were immediately returned to lab,

cleaned of all adhering debris (conifer needles, soil), and dried at 60°C with a circulation fan for 24 hours (larger sporocarps were sliced in half to facilitate drying). One average-sized sporocarp from each collection was weighed to gauge species size. A small portion of the cap was removed for molecular analysis of the fungal ITS region (methodology outlined in Kranabetter *et al*. 2015) to verify guild and species identity through UNITE (Kõljalg *et al*., 2013). The remaining sample (cap and stipe) was ground to < 2 mm for nutrient analysis.

Total C and N concentrations of soil, Douglas-fir foliage and sporocarp tissue were measured using combustion elemental analysis with a Fisons/Carlo-Erba NA-1500 NCS analyzer (Thermo Fisher Scientific, Waltham, MA) (Carter & Gregorich, 2008). Total P (inorganic P + organic P) of mineral soils and forest floors was determined by an ignition method using sulfuric acid and an UV/visible spectrophotometer (O'Halloran & Cade-Menum, 2008). Douglas-fir foliar P was determined by ICP-Atomic Emission Spectroscopy (Teledyne Leeman Labs, Hudson, NH) following microwave digestion on 250 mg tissue. Sporocarp samples, especially for saprotrophic species, were often low in mass so for these P concentration was measured on 50 mg of tissue by ICP-Mass Spectrometry (Agilent Techologies, Santa Clara, CA) following microwave digestion. Both ICP procedures were referenced against two in-house lab standards and the Natural Resources Cananda certified standard DUWF-1.

To facilitate statistical analysis we used soil nutrient content (kg ha^{-1}) of the upper profile (forest floor plus 0-20 cm mineral soil) as a measure of site resources rather than separately testing soil nutrient concentrations by horizon (forest floors were also thin, < 2.5 cm, for many plots). Forest floor concentration data were converted to mass per area using the average depth of the F + H layer at each plot and a bulk density of 0.13 g cm^{-3} (Shaw *et al*., 2005). Bulk density of mineral soil was estimated with a linear model as a function of soil organic matter content (Périé & Ouimet, 2008). Soil C concentrations were converted to soil organic matter using a factor of 0.47 for the 0-20 cm depth. Average coarse fragment content was visually estimated from soil pits and corrected for volume with an assumed specific gravity of 2.6 g cm^{-3}. Nutrient density (kg ha^{-1}) of C, N and P for the upper soil profile was used for determining element ratios (molar basis).

Statistics

Relationships between tissue N and P concentrations by fungal guild and site nutrient content were tested as linear regressions (including a Guild × Soil interaction term) using the GLM procedure in SAS with Type I Sums of Squares (SAS Inc., 2014). The effect of sporocarp size on nutrient concentrations was tested as a covariate with Guild and Soil terms in the GLM. It was not possible to test species effects because of the high turnover in community composition among sites so the residual

error term included the random effect of species nested in guild and site nutrient content. All element ratios were expressed on a molar basis and determined for each foliar/fungal sample before averaging by plot or site type.

The regulatory coefficient H (Sterner & Elser, 2002), as a measure of the plasticity in element ratios, was derived for each guild using plot means for soil and tissue C:N, C:P and N:P ratios. Ratios were log-transformed and the data fit to a linear regression using the GLM procedure in SAS with Type I Sums of Squares (SAS Inc., 2014) to calculate H (= 1/slope), which approaches a value of 1 with decreasing homeostasis (i.e., increasing plasticity) of the fungi (Sterner & Elser, 2002). The difference in log-transformed slopes among guilds was tested pair-wise without adjustment using the estimate statement in the GLM procedure (SAS Inc., 2014).

Results

Soil nutrient status

There was a wide range in soil N and P concentrations across the edaphic gradient as expected. For mineral soils, we found a 5-fold difference in total N% (0.10-0.50) that was well correlated with soil organic matter (soil N% = -0.052+0.047[soil C%]; $p < 0.001$, $r^2 = 0.91$) (Table 1). Inorganic P ranged from over 900 mg kg^{-1} on upland Brunisols to as little as 31 mg kg^{-1} on upland Podzols (Table 1). Organic P was generally less than 250 mg kg^{-1} for upland sites, but almost twice that concentration for lowland Podzols (444 mg kg^{-1}, on average). Overall, the three site types were broadly characterized as low N-high P for upland Brunisols, medium N-low P for upland Podzols, and high N-medium P for lowland Podzols, resulting in a wide divergence in soil C:N:P ratios (Table 2).

Douglas-fir foliar N and P concentrations

Douglas-fir needle N concentrations of the plots ranged from 1.01% to 1.39% across the edaphic gradient, with no significant trend found in relation to soil N content (average 1.24% [SE 0.03]; $p = 0.26$). Foliar P concentrations of Douglas-fir on upland Brunisol plots averaged 0.20% (SE 0.007), and declined sharply across both Podzol site types (0.13% [SE 0.006] for upland Podzols, 0.14% [SE 0.009] for lowland Podzols), resulting, overall, in a significant linear trend with soil P content (foliar P% = 0.11 + 0.000078(soil P [kg ha^{-1}]); $p = 0.010$, $r^2 = 0.44$). Carbon concentrations of the needles averaged 51.6% (SE 0.08).

Sporocarp collections

We made 146 terrestrial sporocarp collections for tissue analysis (89 EMF and 57 saprotrophs collections in total), with an average of 10.4 per plot (range 8-14) that was split between 6.3 EMF and 4.1 saprotroph samples, on average. These collections amounted to 75 fungal species overall,

comprised of 43 EMF and 32 saprotroph species (Supporting Information Table S1). Individual sporocarps were as small as 0.005 g (*Atheniella aurantiidiscsa*) to as large as 5.0 g (*Russula xeramphelina*), with EMF species generally larger than saprotrophic species (average of 0.5 and 0.07 g, respectively).

Sporocarp C, N, and P concentrations

Fungal C concentrations ranged between 37-46%, and were slightly but significantly ($p < 0.001$) lower for saprotroph species on average (41.4% [SE 0.23] and 43.1% [SE 0.16] for saprotroph and EMF sporocarps, respectively). Fungal N concentrations were significantly higher ($p < 0.001$) by almost 40% for saprotrophic species (5.17% [SE 0.26]) over EMF species (3.73% [SE = 0.11]), with no effect of sporocarp size ($p = 0.27$). There was a positive, linear relationship between fungal N% and soil N content ($p < 0.001$), along with a significant interaction by guild ($p = 0.009$) (Fig. 1a). Saprotroph species had greater gains in tissue N% with increasing soil N (4.2%-7.6% on average, slope of 0.00087) compared to EMF species (3.2%-4.7%, slope of 0.00036) (Fig. 2a). Within plots, the range in fungal N% among species of either guild was considerable, equal to an average coefficient of variation (CV) of 24% (21% and 28% for EMF and saprotrophic guilds, respectively).

Fungal P concentrations of saprotroph species were, on average, twice that of EMF species ($p < 0.001$), equal to 1.10% [SE 0.06] vs 0.53% [SE 0.02] respectively, again with no effect of sporocarp size on nutrient concentrations ($p = 0.44$). There was a significant increase in fungal P% with soil P content ($p = 0.011$), but no interaction by guild was detected ($p = 0.30$) (Fig. 1b). The variation in fungal P% was high, however, especially for saprotroph species (average CV of 34% versus 23% for EMF). To illustrate some of these species effects, the average P% of selected sporocarp collections between Brunisol and Podzol sites are shown in Table 2.

Element ratios and regulatory coefficient H

All of the element ratios were significantly lower ($p < 0.001$) for saprotrophs in comparison to EMF. Fungal C:N, C:P and N:P of saprotrophs averaged 10.7 (0.8), 117 (5), and 11.2 (0.5), respectively, while EMF had ratios of 14.4 (0.6), 227 (13), and 16.6 (0.6). The average C:N:P of saprotroph species was 118 (± 15):11 (± 1):1, with a relatively small amount of variation over the edaphic gradient, while EMF species were less nutrient dense at 229 (± 13):17 (± 1):1, on average, and less homeostatic over the edaphic gradient, especially between Brunisol and Podzol sites (Table 2).

The C:N ratio of both fungal guilds and Douglas-fir foliage increased with soil C:N ($p = 0.017$), but we did not detect a guild interaction among the three biota due in part to the low precision of the linear model ($r^2 < 0.4$ for each guild; Fig 2a, Table 3). C:P ratios, in contrast, had a clear interaction among

guilds ($p < 0.001$) as there was essentially no change in C:P of saprotrophs, while EMF and Douglas-fir had large and parallel increases in tissue C:P (Fig. 2b, Table 3). All three guilds demonstrated significant, linear trends in tissue N:P with soil N:P, but EMF was the guild that was most closely aligned (H = 2.9) to the edaphic gradient (Fig. 2c, Table 3). Overall, we found a high degree of symmetry (nearly an identical 1:1 relationship) between EMF sporocarp N:P and Douglas-fir foliar N:P over the study sites (Fig. 3).

Discussion

The strongly podzolized soils of temperate rainforests along the west coast of British Columbia were relatively C and N rich but greatly limited in inorganic P compared to less-weathered Brunisol soils, consistent with patterns in podzol development reported elsewhere (Turner *et al*., 2012). Consequently, the wide range in soil C:N:P under a single tree species was an ideal environmental gradient to elucidate patterns in fungal stoichiometry. A notable difference between fungal guilds was the much lower concentrations (on average) of both N and P for EMF compared to saprotrophic sporocarps, as we had hypothesized, which is consistent with the primary function of fungal symbionts as nutrient conduits in support of trees. A substantial depletion in symbiotic fungal N% via transfer to the host would also be consistent with the greater extent of N isotope fractionation commonly found in EMF sporocarps compared to saprotrophs (Mayor *et al*., 2009). We can confirm a degree of homeostasis in element ratios for fungi (H > 1) as there are limits in the physiological malleability of these organisms, but a fully constrained response to nutrient availability was uncommon (Fanin *et al*., 2013; Danger & Chauvet, 2013; Gulis *et al*., 2017). Interestingly, the median fungal C:N:P ratio of 250:16:1 reported in a meta-analysis by Zhang & Elser (2017) was more aligned with EMF (229:17:1) than saprotrophs (118:11:1) in our study, possibly due to the predominance of EMF biomass in many of the forest soils previously studied.

The significant gains in tissue N% and narrowing of C:N ($H \sim 2.5$) by free-living saprotrophs and symbiotic fungi over the edaphic gradient was evidence of weak homeostatic regulation for N by both guilds (largely consistent with Vogt *et al*., 1981; Trudell & Edmonds, 2004). We attribute this to two mechanisms described by Mooshammer *et al*. (2014); a physiological adjustment of microbes to enhance storage of elements in excess, and, perhaps more importantly, community turnover to accommodate those species most effective in resource exploitation. For N resources this includes mobilization and direct uptake of organic N on low fertility sites, in contrast to the dominance of inorganic N forms across richer ecosystems. Kranabetter *et al*. (2015) demonstrated this adaptation to site in the enhanced uptake capacity of NH_4^+ among EMF species dominating similarly rich coastal ecosystems. An ability to scale up N acquisition with soil N availability likely reflects an appreciable level

of competition for N between guilds across a full gradient in soil fertility, which could be of interest in models of soil C storage (Orwin *et al*., 2011; Bödeker *et al*., 2016; Philpott *et al*., 2018) and consumer-driven nutrient recycling (Zechmeister-Boltenstern *et al*., 2015). In addition, a less constrained response in fungal N% could have implications for related processes such as decomposition rates (Koide & Malcolm, 2009) and C flux (Trocha *et al*., 2010). It was also interesting to note the convergence between guilds in tissue N% on sites with the lowest N content (Fig. 1a). We surmise that the amount of N available for transfer by the symbiotic fungi to the host would be nil if these regression lines ever intersected, indicating soil N capital so limited that the ecosystem could not support anything beyond fungal biomass.

The sharp decline in foliar P% of coastal Douglas-fir to levels of deficiency (< 0.15%, Carter, 1992) for both upland and lowland Podzols emphasized how P is a pervasive and fundamental constraint (or, at the very least, co-limiting with N) across these temperate rainforests (Blevins *et al*., 2006). There was clear symmetry in C:P between EMF and Douglas-fir (Fig 2b), in contrast to the more constrained C:P of saprotrophs. This finding nullified our hypothesis of homeostatic behaviour by fungal symbionts; instead, the results indicate an equally shared constraint in P among EMF communities and host trees, with neither partner benefiting from enhanced nutrition at the expense of the other. Perhaps such an equal relationship is in keeping with the mutualistic nature of a 'holobiont', which is characterized by strongly interlinked biota rather than autonomous individuals maximizing their own fitness (Bordenstein & Theis, 2015; Vandenkoornhuyse *et al*., 2015). There was less symmetry in tissue N, however, because Douglas-fir foliar N% was essentially unchanged over the edaphic gradient in contrast to EMF. The lack of a trend in foliar N relative to soil N for trees could be considered atypical (Littke *et al*., 2014), but given the overriding constraints of P in this environment it is possible the trees allocated N to other sinks such as root biomass (Ostonen *et al*., 2017).

A second key finding regarding P was the marginal change in sporocarp P% and strong homeostasis in C:P of saprotrophic fungi on strongly podzolized soils. To be clear, within saprotrophic communities there was considerable variability in P%, and the litter-decay fungi (e.g., *Mycena*), not surprisingly given their growth on conifer needles, were generally low in P%. However, other saprotrophic species more widely distributed through the soil profile, such as *Clitocybe*, *Entoloma* (formerly *Nolanea*) and *Cantharellula*, had consistently high concentrations of P over the entire edaphic gradient (~ 1.8%; Table 2). Is it possible, then, that some free-living saprotrophic fungi have superior abilities in the mobilization and uptake of organic or occluded P compared to EMF? The lack of lignocellulolytic enzymes by some lineages of EMF, for example, may limit P liberation from organic

substrates, as has been speculated for organic N acquisition (Pellitier & Zak, 2018). Likewise, Talbot *et al*. (2013) found that P-targeting enzymes were better correlated with saprotroph community structure, while Teste *et al*. (2016) reported declining extraradical hyphal biomass on P impoverished soils. We would, however, temper these suppositions with the recognition of profound differences in biomass among the free-living and symbiotic fungal guilds. Saprotrophic fungi are discrete individuals, comprised of vegetative hyphae with a limited distribution, while EMF are intimately connected with the entire autotrophic biomass of host trees (> 35 m tall for most of these stands). Even if P mobilisation and acquisition traits were identical between fungal guilds, the inherently limited supply of P from podzolized soils would be stretched much further in a holobiont of dramatically greater size. This possible mismatch in scale between free-living and symbiotic fungi hinders some interpretations of stoichiometry, so at this point we only emphasize how critical the effectiveness of P acquisition by EMF must be to temperate rainforest ecology.

Element ratios of foliar N:P can provide a useful index of nutrient limitations (e.g., N limitation < 14 versus P limitation > 16; Cleveland & Liptzin, 2007) and substantiate evidence of intensive soil weathering and possibly ecosystem decline (Wardle *et al*., 2004). Here we can extend these interpretations to terrestrial fungi. While both fungal guilds (and host trees) displayed significant trends in tissue N:P in relation to soil N:P, the utility of this index was most evident for EMF. Saprotrophs had much lower N:P ratios, on average, than EMF (consistent with Zhang & Elser, [2017]), and a greater degree of homeostasis over the edaphic gradient (Fig. 2c) due to their enhanced P nutrition on podzolized soils. In contrast, N:P ratios of EMF displayed a very similar “breakpoint” to foliar N:P in regards to P limitations (< 12 on Brunisols, > 16 on Podzols) and was more strongly and consistently aligned with soil N:P (H = 2.9). There was a remarkable, nearly identical symmetry in N:P ratios between EMF sporocarps and Douglas-fir foliage across the edaphic gradient (Fig. 3), despite the completely different cell structure between fungal hyphae and conifer needles. This fidelity in N and P stoichiometry reinforces our premise of site constraints shared in equal measure between EMF and host trees, which ultimately might be more conducive to maximizing fitness of the holobiont.

Considerable variation existed in sporocarp N% and P% among fungal species within plots that likely arose from a number of factors. One source would be microsite heterogeneity, especially where decayed wood was fully incorporated into the forest floor, although we attempted to minimize this effect by gathering scattered individual sporocarps for each collection. Morphology of the sporocarps, such as the amount of cap tissue relative to the stipe, could also contribute to the variation in nutrient concentrations among species (Trocha *et al*., 2016). Of most interest, however, would be if the variation

among species accurately reflects traits pertaining to N and P uptake effectiveness. For example, how might P acquisition compare between co-occurring but disparate EMF species (Table 2) such as *Helvella vespertina* (1.2%) and *Russula xeramphelina* (0.4%)? Such wide contrasts in species nutrition may be evidence of niche partitioning within communities (Turner, 2008), and would be quite plausible given the well documented range in functional traits among fungal species for both N and P acquisition (e.g., Zhang *et al*., 2014; Walker *et al*., 2014). Some nutrient disparity among fungal species may also reflect contributions made by associated bacteria of the mycorrhizosphere (Calvaruso *et al*., 2007; Brooks *et al*., 2011; Fontaine *et al*., 2016). Evaluations of species niche and community assembly processes would be greatly facilitated if measured functional traits paralleled rankings in sporocarp nutrition.

The fidelity in which sporocarp stoichiometry reflects that of vegetative mycelium remains an open question. Resolving this issue for terrestrial fungi is almost intractable because of the difficulties in isolating hyphae of separate guilds and species from bulk soils, and the challenges in culturing fungal mycelium (especially EMF species) to include an induction of fruiting for analysis. As way of comparison, aquatic fungal mycelium display a similar capacity for non-homeostatic response to nutrient availability (Danger *et al*., 2016), and elemental composition of some aquatic hyphomycetes align well with our saprotrophic sporocarps (e.g., N:P ratio ranging from 11-16; Grimmett *et al*., 2013). The possible effect of hyphal growth rates (Gulis *et al*., 2017) on sporocarp stoichiometry should be minimal as primordium are set earlier in the season and will expand, rather than add new hyphae, upon the initiation of fruiting in the fall. . One concern with the study methodology was that sporocarp mass might confound nutrient patterns (i.e., larger sporocarps would have more diluted N and P concentrations) but there was no evidence of a size effect in our analysis.

Despite the rich species diversity of fungal communities on the west coast of B.C. (Roberts *et al*., 2004), we were able to detect distinct trends in community response with a reasonable effort in sampling. It should be noted, however, that many important genera (e.g., *Piloderma*, *Tomentella*) are excluded under this methodology because they lack conspicuous fruiting bodiesSoil resource availability was based upon total C, N and P content, which was a logical starting point for elucidating stoichiometry patterns and a common baseline for global-scale analysis. Patterns in fungal nutrition and stoichiometry could possibly be further refined with more detailed measures of soil resources, such as the Hedley fractionation of soil P (Johnson *et al*., 2003). Likewise, we found soil C:N to be more ambiguous in portraying guild response, particularly for saprotrophs, than soil N capital. This may be because of less distinction in soil C:N across the edaphic gradient, such that Podzols with a high N and C content would rank closely to Brunisols (in terms of C:N) but nevertheless have greater amounts of mineralized N for

uptake. More precise methods of determining soil N availability, such as *in situ* buried bags, may further clarify the extent of homeostasis in fungal N% and C:N between guilds.

Conclusions

For decades, questions concerning nutritional effects of mycorrhizae have focused on plants with or without mycorrhizal fungi (Corrêa *et al*., 2012). Here we offer an alternative perspective; what is the nutritional status of fungi with or without a host? The comparison is apt because of the shared ancestry and considerable overlap in functional traits among many free-living and symbiotic fungi. We found distinct nutrient concentrations and element ratios of sporocarps between fungal guilds, and significant interactions across soil nutrient gradients highlight their contrasting roles in forest ecosystems. The primary function of EMF as nutrient conduits was underscored by the close alignment of fungal C:P with host trees. A high degree of symmetry and almost identical scaling was also found in tissue N:P between EMF and Douglas-fir, demonstrating how these nutrient constraints appear well balanced throughout the holobiont. We conclude that the empirical evidence provided by fungal sporocarp stoichiometry will be a valuable tool to further advance our understanding of the structure and function of mycorrhizal relationships (Johnson, 2009), the nature and extent of competitive interactions between fungal guilds (Fernandez & Kennedy, 2016), and the adaptive response of fungal communities to environmental gradients and ecosystem development (Dickie *et al*., 2013).

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Author contributions: JMK and BJH conceived study; JMK, R H-D and BJH collected field data; JMK and R H-D analyzed data; and JMK led the writing with substantial contributions from R H-D and BJH.

Data accessibility: all fungal nutrient analysis, soil chemistry, and foliar nutrition will be made available at Dryad upon acceptance of the manuscript. Sequence data deposited at UNITE under accession numbers UDB034777-UDB034848.

Supporting Information

Figure S1. Forest ecosystem and soil profile images.

Table S1. Saprotrophic and ectomycorrhizal fungal species.

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590 Table 1. Plot location and site features, including mineral soil (0-20 cm) concentrations of total C, N, and P (inorganic P_i and organic P_o) (plot
591 mean and standard error in brackets, n = 3).

Plot	Latitude (N)	Longitude (W)	Elev. (m)	MAT* (°C)	MAP (mm)	Soil C (%)	Soil N (%)	Soil P_i (mg kg^{-1})	Soil P_o (mg kg^{-1})
Upland Brunisols									
Salt Spring	48°44'33"	123°29'10"	400	8.2	1110	2.8 (0.4)	0.10 (0.01)	621 (38)	179 (34)
Kapoor	48°27'54"	123°36'15"	310	8.7	1335	3.6 (0.4)	0.11 (0.01)	286 (74)	137 (12)
Sooke	48°33'30"	123°42'53"	260	8.9	1615	4.3 (0.9)	0.10 (0.01)	555 (137)	138 (60)
Niagara	48°28'31"	123°34'34"	400	8.3	1441	5.1 (0.9)	0.19 (0.02)	959 (28)	177 (17)
Mt Prevost	48°50'21"	123°44'20"	200	9.0	1348	3.3 (0.2)	0.16 (0.01)	903 (126)	233 (25)
Upland Podzols									
WC 1000	48°33'12"	124°21'02"	250	8.1	3415	5.7 (0.6)	0.22 (0.03)	46 (14)	163 (46)
Fairy Lk	48°35'55"	124°19'18"	300	8.0	3529	8.4 (1.0)	0.29 (0.03)	51 (2)	205 (14)
Br. 167 (1)	48°54'51"	124°49'21"	220	8.4	3538	5.7 (0.2)	0.20 (0.01)	235 (23)	290 (15)
Br. 247 (1)	48°51'00"	124°53'02"	265	8.2	3569	4.9 (0.4)	0.15 (0.01)	31 (9)	134 (11)
Lowland Podzols									
San Juan	48°35'17"	124°11'59"	60	8.7	3066	7.7 (0.9)	0.28 (0.04)	405 (105)	353 (40)
Br. 136	48°53'52"	124°54'41"	140	8.8	3186	11.3 (0.3)	0.50 (0.02)	103 (18)	401 (29)
Br. 167 (2)	48°54'51"	124°49'21"	220	8.4	3538	8.2 (0.7)	0.38 (0.03)	93 (11)	513 (18)
Br. 247 (2)	48°51'00"	124°53'02"	265	8.2	3569	6.0 (1.5)	0.25 (0.06)	136 (10)	416 (80)
Klanawa	48°49'11"	124°46'29"	95	8.9	3259	7.0 (0.4)	0.32 (0.02)	89 (35)	535 (35)

592 * mean annual temperature (MAT) and precipitation (MAP) for the 30-yr period 1961-1990 were obtained for each location by querying ClimateWNA ver 4.72
593 (Wang et al. 2012) with latitude, longitude and elevation.

Table 2. Average element ratios (molar) of soil and guild fungal tissue by site type (95% confidence limits in brackets), along with phosphorus concentrations of selected fungal species among Brunisol or Podzol sites.

Site type	**Soil profile** C:N:P	**N***	**Saprotroph** C:N:P	**N**	**Ectomycorrhizae** C:N:P
Upland Brunisols	150 (± 53):4 (± 1):1	27	122 (± 25):10 (± 2):1	30	177 (± 18):11 (± 1):1
Upland Podzols	896 (± 338):23 (± 8):1	13	121 (± 28):13 (± 3):1	25	269 (± 18):20 (± 2):1
Lowland Podzols	429 (± 119):15 (± 5):1	17	112 (± 25):12 (± 1):1	34	245 (± 16):19 (± 2):1
Brunisols	**Species**		**%P** (SAP ave. = 1.09%)		**%P** (EMF ave. = 0.67%)
	Clitocybe deceptiva	5	1.75 (± 0.15)		
	Cystoderma granulosum	3	1.04 (± 0.07)		
	Mycetinis salalis	4	0.67 (± 0.08)		
	Helvella vespertina			2	1.23 (± 0.03)
	Hebeloma crustuliniforme			4	0.76 (± 0.13)
	Inocybe lilacina			3	0.65 (± 0.05)
	Russula xeramphelina			3	0.44 (± 0.03)
Podzols (Upland and Lowland)	**Species**		**%P** (SAP ave. = 1.11%)		**%P** (EMF ave. = 0.47%)
	Entoloma cetratum	3	1.91 (± 0.17)		
	Cantharellula umbonata	4	1.77 (± 0.07)		
	Atheniella aurantiidiscsa	4	0.95 (± 0.08)		
	Phaeocollybia sp.			2	0.75 (± 0.01)
	Lactarius hepaticus			6	0.58 (± 0.05)
	Cantharellus formosus			4	0.40 (± 0.04)
	Clavulina corraloides			7	0.38 (± 0.04)

* number of sporocarp collections per site type or by species

598 Table 3. Model outputs and H coefficient for element ratios by guild (saprotrophic fungi [SAP],
599 ectomycorrhizal fungi [EMF], Douglas-fir foliage [TREE]) in relation to soil (n = 14 per guild, all data log-
600 transformed as depicted in Figure 2).

Consumer and Resource	Guild	General Linear Model	Coefficient H
Tissue C:N and soil C:N	SAP	= 0.45+0.37(soil C:N); r^2 = 0.09	2.7
(Guild × Soil p = 0.68)	EMF	= 0.47+0.45(soil C:N); r^2 = 0.39	2.2
	TREE	= 1.43+0.17(soil C:N); r^2 = 0.20	5.9
Tissue C:P and soil C:P	SAP	= 2.15 – 0.03(soil C:P); r^2 = 0.02	33a*
(Guild × Soil p < 0.001)	EMF	= 1.75 + 0.24(soil C:P); r^2 = 0.87	4.2b
	TREE	= 2.38 + 0.22(soil C:P); r^2 = 0.66	4.5b
Tissue N:P and soil N:P	SAP	= 0.89+0.17(soil N:P); r^2 = 0.43	5.9a
(Guild × Soil p = 0.046)	EMF	= 0.85+0.35(soil N:P); r^2 = 0.83	2.9b
	TREE	= 0.99+0.26(soil N:P); r^2 = 0.70	3.8ab

601 * significant differences (p < 0.05) in coefficient H among guilds for each nutrient separated by letters.

602

Fig. 1. Linear patterns in fungal tissue nitrogen [a] and phosphorus [b] concentration (%) by guild (dotted line SAP = saprotrophic fungi, dashed line EMF = ectomycorrhizal fungi) as a function of soil nutrient content (kg ha^{-1}).

Fig. 2. Average fungal and foliar tissue element ratios (C:N in [a], C:P in [b], N:P in [c]) as a function of the soil element ratios for the derivation of the regulatory coefficient H (n = 14 for each guild; all data log-transformed). EMF = ectomycorrhizal fungi (gray diamonds, dashed line); SAP = saprotrophic fungi (white squares, dotted line); TREE = Douglas-fir foliage (gray triangles, dot and dash line). A 1:1 relationship between tissue and soil nutrient ratios (identical stoichiometry) is depicted by the solid gray line.

Fig. 3. Relationship in average N:P ratio between EMF sporocarps and Douglas-fir foliage over the 14 study sites (mean ± 1 SE for each guild; 1:1 relationship depicted by solid gray line). Slope of the linear regression = 0.86, r^2 = 0.89.