Estimation of fungal biomass in forest litter and soil

Petr BALDRIAN*, Tomáš VĚTROVSKÝ, Tomáš CAJTHAML, Petra DOBIÁŠOVÁ, Mirka PETRÁNKOVÁ, Jaroslav ŠNAJDR, Ivana EICHLEROVÁ

Institute of Microbiology of the ASCR, Vídeňská 1083, 14220 Praha 4, Czech Republic

ABSTRACT

The contents of fungal biomass markers were analysed in the fruit bodies of dominant basidiomycetes from an ectomycorrhiza-dominated coniferous forest, and used to estimate the fungal biomass content in the litter and soil. The content of ergosterol (3.8 ± 2.0 mg g^{-1} dry fungal biomass) and the phospholipid fatty acid 18:2ω6,9 (11.6 ± 4.3 mg g^{-1}) showed less variation than the internal transcribed spacer (ITS) copy numbers ($375 \pm 294 \times 10^9$ copies g^{-1}). A high level of variation in the ITS copy numbers (per ng DNA) was also found among fungal taxa. The content of fungal biomass in the litter and soil, calculated using the mean contents, varied between 0.66 and 6.24 mg g^{-1} fungal biomass in the litter, and 0.22 and 0.68 mg g^{-1} in the soil. The ratio of fungal biomass in the litter to that in the soil varied greatly among the markers. The estimates of fungal biomass obtained with different biomarkers are not exactly comparable, and caution should be used when analysing taxon abundance using PCR amplification of fungal rDNA.

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Introduction

Forest ecosystems in the boreal and temperate biomes typically have a high content of carbon stored on the soil surface in the form of plant litter. In the soil, carbon accumulates as a result of both litter decomposition and the deposition of photosynthesis-derived carbon by tree roots. Fungi are an important part of these ecosystems, and their biomass is high; the ectomycorrhizal biomass alone has been estimated to be as high as 700—900 kg ha^{-1} in a coniferous forest (Wallander et al. 2001). Fungi in the forest floor are represented by both the saprotrophs, which decompose litter and soil organic matter, and the ectomycorrhizal fungi, which are dependent on association with tree roots. While the former prevail in the litter, where the availability of hydrolysable compounds supports fast decomposition and ultimately mineralisation of carbon (Lindahl et al. 2007; Šnajdr et al. 2011a), the latter typically dominate in the soil environment, although they may also be quantitatively important in the litter (as is the case of the Norway spruce (*Picea abies*), which has a shallow rooting system) (Baldrian et al. 2012).

Comparison of the composition of fungal communities in the litter and various soil horizons and the identification of important taxa are currently achievable with the use of high-throughput sequencing methods, but a complete understanding of the ecology of forest ecosystems is only possible if the fungal biomass in the litter and soil can be reliably quantified and compared.

In 2008, Joergensen & Wichern reviewed the methods for the quantification of fungal biomass in soils, and they distinguished among the following methods: (1) microscopic techniques, (2) selective respiratory inhibition, (3) quantification of cell membrane components (ergosterol and phospholipid fatty acids; PLFA) and (4) quantification of cell wall components (glucosamine in fungi). The use of methods (1), (2) and (4) has declined because the microscopy-based approaches show high variation in their estimates and tend to deviate from other methods, respiration methods are difficult to use for large sample sites and the glucosamine content in fungal mycelia is variable and is often associated with dead biomass or organic soil matter (Appuhn & Joergensen 2006; Joergensen & Wichern 2008). In contrast, methods based on DNA quantification receive increasing attention, not only for their ability to estimate fungal biomass content but also because species composition data can be provided (e.g., by high-throughput sequencing). The aim of this paper was to compare the dominant methods currently used for fungal biomass estimation in forest ecosystems: the quantification of ergosterol and PLFA with the use of quantitative PCR (qPCR). While previous studies have relied mainly on soil analysis, we have also applied these methods to the litter, a horizon rich in fungal biomass, to explore the differences between the fungal biomass in litter and soil. These two environments differ in fungal abundance, community composition and the contents of decomposition-related enzymes (Joergensen & Wichern 2008; Šnajdr et al. 2008; Baldrian et al. 2012). We used the same set of fungal taxa for the quantification of the three common biomarkers in fruit bodies and applied the same extraction procedures that are used in soil and litter to derive conversion factors allowing estimation of fungal biomass content. The study was performed in a mountainous spruce (P. abies) forest dominated by ectomycorrhizal fungi (Baldrian et al. 2012), and fungal fruit bodies representative of the area of study were used as the analysed material.

Some studies have shown that the content of fungal biomarkers depends on the available nutrients (Charcosset & Chauvet 2001). It is thus possible that the content of these biomarkers in fungal fruit bodies might be similar to that of mycelia growing in soil, which also use root- or soil-derived nutrients. These are largely different from the nutrients used for the production of fungal biomass in vitro, which has typically been used for such analyses (Joergensen & Wichern 2008). Furthermore, the use of dominant fungal fruit bodies should give a better representation of the true biomarker content than laboratory cultivatures because many ectomycorrhizal (ECM) fungi do not grow in culture and their mycelia thus cannot be produced in vitro. Consequently, this approach should yield better conversion factors between fungal biomarker content and biomass as well as a better estimate of variation among taxa. Of course, the use of fruit bodies also has limitations because many fungi, most notably the saprotrophic microfungi, are unavailable in this form, and the properties of fungal biomass in the fruit bodies may differ from that of the soil mycelia.

Although some studies have used qPCR to enable relative comparisons of fungal biomass content between soil or litter samples (e.g., Baldrian et al. 2012; Ihrmark et al. in press), here we present one of the first attempts to calculate the conversion factors between the rDNA content and fungal biomass. The question about the potential applicability of this approach is important with respect to the current development of molecular methods for fungal quantification using qPCR and community analysis. With the latter in mind, we have also explored the variation in fungal rDNA (ITS) copy numbers across fungal taxa because the quantification of these molecules is frequently used for the relative quantification of individual fungal taxa in the soil community. Although some studies show that such estimates might be inappropriate for several reasons, including DNA extraction, PCR efficiency and method-specific problems (Amend et al. 2010), the extent to which such results are affected by the differences in the ITS contents in fungal biomass remain unexplored.

Materials and methods

Collection of litter, soil samples and fungal fruit bodies

The study area is located in the highest altitudes (>1 100 m above sea level) of the Bohemian Forest National Park (Central Europe: 49°02.64 N, 13°37.01 E), and is covered by an unmanaged spruce (P. abies) forest. Sampling was performed in early Oct. Six topsoil profile samples were collected at four sites within an area of 25 m^2 that were located approx. 250 m from each other. Litter horizon (L) and organic soil (S) material were separately pooled. After the removal of roots, L material was cut into 0.5 cm pieces and mixed; S material was passed through a 5-mm sterile mesh and mixed. Aliquots for use in nucleic acid extraction were immediately frozen and stored in liquid nitrogen. Samples of soil were immediately frozen in liquid nitrogen and stored on dry ice. Samples for DNA extraction, PLFA and ergosterol analysis were stored at −45 °C. The soil dry mass content was measured after drying at 85 °C, the organic matter content was determined following burning at 650 °C, and pH was measured in distilled water (1:10). Soil C and N contents were measured using an elemental analyser.

Fungal fruit bodies were collected from the summer to the autumn of 2009 and 2010 in and around the area of sampling. For the eleven species that showed the highest fruit body abundance in the studied area (Table 1, Supplementary Table 1), three independent samples of fresh fruit bodies were collected and analysed. Each sample consisted of either a single stipe of a large fruit body or, in the case of smaller fungi, several fruit bodies growing close to one another (<50 cm). Soil and attached objects were cleaned from fungal fruit bodies in the forest, and when possible, inner parts of the fruit bodies were excised and the rest was discarded. The fruit bodies were immediately refrigerated and stored frozen at −45 °C until the extraction of DNA, PLFA and ergosterol. The dry mass content of fruit body subsamples was measured after freeze-drying. For the analysis of fungal DNA, twenty-four additional fungi that occurred less frequently in the study area (Supplementary Table 1) were also analysed. The samples were treated as above and stored frozen at −45 °C until they were processed for DNA extraction.