Cut *Picea abies* Stumps Constitute Low Quality Substrate for Sustaining Biodiversity in Fungal Communities

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Abstract

As large-scale stump harvesting for biofuel is implemented in North Europe, it raised concern of depriving of coarse dead wood and reducing biodiversity of wood fungi. Aims of this study: i) to analyze what sampling effort would be required to reflect fungal diversity in cut tree stumps; ii) to evaluate relevance of found species for nature conservation. A total of 300 wood samples were taken from 60 stumps at a clear cut area, cultures of fungi isolated and subjected to molecular identification. In total, 839 of fungal strains were obtained (approx. 3 per sample and 14 per stump), representing 51 taxa. The extent of sampling has largely reflected species richness of (cultivable) fungi in stumps and on the clear-cut. Conclusions: i) among detected all were common fungi; ii) cut stumps commonly and regularly harbor important tree pathogens, representing bases for their establishment, reproduction and further spread; iii) no threatened species of fungus has been ever reported to colonize and complete lifecycle on a cut logging stump; iv) stump harvesting does not pose any threat for rare and vulnerable fungi of natural forest.

Keywords: wood decomposition, coarse woody debris, wood harvesting, basidiomycetes, ophiostomoid fungi.

Introduction

Presence of coarse woody debris (CWD: snags and fallen logs > 10 cm in diameter) in Fennoscandian forests is of crucial importance for many threatened, i.e. red-listed, saproxylic organisms (Siitonen 2001). In particular this includes wood-inhabiting fungi. Extensive studies in the region have demonstrated that communities of those fungi differ significantly between managed stands and natural forests, and that threatened species almost exclusively occur on large dimension CWD at advanced stages of decay (Penttilä 2004, Penttilä et al. 2004).

Harvesting of tree stems has the most profound impact on CWD availability in forest ecosystems, and in Fennoscandia it might reduce the average amount of CWD at the landscape level by 90-98% (Siitonen 2001). In such instance, cut tree stumps in managed forests become increasingly dominant type of large-diameter deadwood substrate (or CWD). It has been estimated that on clear-cuts where slash has been removed, stumps may comprise up to 80% of remaining CWD (Egnell et al. 2007) and around 75% of the total production of CWD during a managed forest's rotation (Dahlberg et al. 2011). According to existing data, stump and coarse roots of a tree constitute approx. 20% of stem biomass (Lundmark 1988, Hakkila 1989). Thus, following harvesting 250 m^3 ha^{-1} of stemwood, volume of left stumps and coarse roots on a clear-cut would constitute approx. 50 m^3 ha^{-1}.

Whole this amount of deadwood is to be colonized, utilized and decomposed by wood-inhabiting fungi, and in many instances would also serve as a basis for completion of their lifecycles, in other words, sporocarp formation and spore dispersal. To date, available studies on fungi in cut stumps of trees (focused mostly on *Picea abies*) revealed highly diverse communities (Vasiliauskas et al. 2002, 2004, 2005, Arhipova et al. 2011). As large-scale

stump harvesting for bioenergy purposes is currently being implemented in Sweden and Finland (Björheden 2006, Saksa 2013), this raised the concern that stump removal and the subsequent reduction of CWD may negatively affect biodiversity of wood-inhabiting organisms, including fungi (Egnell et al. 2007, Walmsley and Godbold 2010). Moreover, although little is known on possible direct impact of stump harvesting on soil conditions, majority of available related studies indicated insignificant or even positive effect of de-stumping on growth and mycorrhization on such sites of subsequently replanted seedlings (Vasaitis et al. 2008, Klavina et al. 2016).

To date, only a single study has been conducted to examine impacts of stump harvesting on biodiversity of wood-inhabiting fungi (Toivanen et al. 2012). Sporocarp surveys of polypores and agarics were accomplished on 20 *P. abies*-dominated clear-cuts, 10 of which were subjected to stump and slash removal, and 10 were left as controls. A total of 22 870 sporocarps were observed which belonged to 148 taxa, and authors concluded that stump removal has a negative effect on fungal diversity. It must be noted, however, that none of the observations included a record of a threatened species.

The results of numerous studies show that diversity and volume of dead wood are the most important variables for species richness of red-listed polypores (Hottola and Siitonen 2008, Junninen and Komonen 2011). The decrease in diversity in communities of all macrofungi (observations of 1413 taxa) has been reported on forest sites with increasing intensity of management (Hofmeister et al. 2014). As cut stumps represent uniform dominating CWD substrate and are the most profound attribute of managed forests, their roles for supporting fungal biodiversity of natural forests therefore might be of limited value, although are yet not fully understood.

Therefore, more studies in this respect are needed, that would comprise also other groups of fungi (e.g. corticioids and microfungi) and explore in more detail their occurrence in stumps on harvested sites, e.g. by using pure culture isolations (Arhipova et al. 2011) or molecular detection of fungi directly from wood samples (Kubartova et al. 2012, Ovaskainen et al. 2013). As stumps of mature trees represent relatively large resource units, while taking samples for isolation or direct detection it would be valuable to know what sampling effort (how many samples from what size of stumps) would be needed and reasonable in order to reasonably reflect diversity of fungal communities. The aims of this study were: i) first, to analyze what sampling effort would be required to reflect fungal community inhabiting cut tree stump, and ii) second, to discuss the value of cut stumps for threatened species in light of results of this and other related studies.

Materials and Methods

Study area

The study site was located at the Hyytiälä Forest Field Station of the University of Helsinki (N61°51'', E24°17'', 160 m a.s.l.) in central Finland. Initially the area represented boreal coniferous forest dominated by 100-year-old *P. abies* with admixture of *Pinus sylvestris* and *Betula* spp. of *Oxalis-myrtillus* site type. It was clear-felled in November 2004.

Stumps and sampling

In June 2008, sixty stumps were randomly selected, numbered and their diameters measured: after selecting the first stump at the edge of the clear-cut, the next closest stump was selected, and so on. Neither of the stumps showed symptoms of pre-established butt-rot, implying that, when cut, trees were decay-free. Each of the stumps was sampled five times (in period of two days) by taking bore cores using increment borer. The borer was inserted approx. 8 cm deep into stump at approx. 3 cm distance from the stump surface. From the same stump the samples were taken possibly distantly from each other. Immediately after the sampling, bore cores were individually placed into sterile Falcon tubes and brought to the laboratory.

Isolation and identification of fungi

Isolation was done the same or next day after sampling, and the procedures closely followed those employed in our earlier study (Arhipova et al. 2011). Each bore core was flame sterilised, individually placed in 9-cm Petri dishes on Hagem agar medium (Vasiliauskas and Stenlid 1998), and incubated in the dark at room temperature. The dishes were inspected on a daily basis and discrete colonies growing out were subcultured to separate dishes. Subsequently, all pure cultures were examined under the microscope and grouped into mycelial morphotypes. From those, certain taxa were identified microscopically based on mycelial morphology (Table 1).

One to three representatives from the rest of mycelial morphotypes were subjected to molecular identification following procedures from the above-cited study (Arhipova et al. 2011). In brief, DNA extraction and PCR amplification were made accordingly to established protocols. The ready PCR products were purified, using Calf Intestine Alkaline Phosphate (CIAP) and *Escherichia coli* exonuclease I. After purification, PCR products were Sanger sequenced by Macrogen using the primer ITS4 for every DNA specimen. The sequencing was performed on one direction. All sequences were manually edited using the Lasergene software package SeqMan. BLAST searches were performed using two reference sequence databases –

Table 1 Fungi isolated from 4-year-old *Picea abies* stumps on clear-cut of a forest stand

Fungi	GeneBank accession no.	% colonized (no. examined)		% in all isolates (n=839)
		Stumps (n=60)	Samples (n=300)	
Basidiomycetes				
Bjerkandera adusta	GU067733	16.7	4.3	1.5
Ceratobasidium sp. 257	GU067737	3.3	1.0	0.4
Cerrena unicolor	GU067741	1.7	0.3	0.1
Cylindrobasidium evolvens	GU067739	10.0	2.3	0.8
Gloeophyllum sepiarium	GU067756	5.0	1.3	0.5
Fomitopsis pinicola	GU067743	18.3	6.0	2.1
Heterobasidion parviporum	- [a]	1.7	0.3	0.1
Hypholoma capnoides	GU067745	3.3	0.7	0.2
Lenzites betulina	GU067734	6.7	1.3	0.5
Peniophora incarnata	GU067740	16.7	5.0	1.8
Phlebiopsis gigantea	- [a]	23.3	9.7	3.4
Resinicium bicolor	- [a]	1.7	0.3	0.1
Sistotrema brinkmannii	GU067742	83.3	41.0	14.4
Stereum sanguinolentum	- [a]	46.7	15.0	5.4
Trametes versicolor	GU067736	3.3	0.7	0.2
Trametes zonata	GU067738	21.7	5.3	1.9
All basidiomycetes		96.7	71.0	33.8
Ascomycetes & deuteromycetes				
Ascocoryne cylichnium	GU067753	35.0	10.7	3.8
Ascocoryne sarcoides	- [a]	16.7	3.3	1.2
Cadophora fastigiata	GU067761	5.0	1.0	0.4
Cadophora malorum	GU067760	5.0	1.0	0.4
Chaetomium globosum	GU067749	1.7	0.3	0.1
Cosmospora vilior	GU067755	31.7	7.7	2.7
Drechslera sp. F72	GU067763	1.7	0.3	0.1
Gibberella avenacea	- [a]	5.0	1.3	0.5
Grosmannia cucullata	GU067758	13.3	3.3	1.2
Grosmania olivacea	GU067766	15.0	4.3	1.5
Grosmania piceaperda	GU067757	6.7	1.7	0.6
Hormonema dermatioides	- [a]	58.3	17.7	6.3
Hypocrea pachybasioides	KJ742593	18.3	5.3	1.9
Lecytophora sp. F47	GU067748	30.0	6.7	2.4
Lecytophora sp. F66	GU067759	1.7	0.3	0.1
Leptodontidium elatius	GU067735	8.3	2.0	0.7
Mariannaea elegans	GU067754	16.7	5.3	1.9
Neonectria fuckeliana	- [a]	56.7	19.0	6.8
Nectria sp. F58	GU067752	1.7	0.3	0.1
Neonectria ramulariae	GU067762	5.0	2.0	0.7
Ophiostoma piceae	GU067767	51.7	14.3	5.1
Penicillium spinulosum	GU067750	1.7	0.3	0.1
Penicillium sp.	- [a]	45.0	14.3	5.0
Phialocephala fortinii	GU067764	1.7	0.3	0.1
Rhinocladiella atrovirens	KJ742592	3.3	0.2	0.2
Rhinocladiella sp. F74	GU067765	1.7	0.3	0.1
Trichoderma viride	GU067751	38.3	18.3	6.6
Unidentified sp. F42	GU067746	3.3	0.7	0.2
Unidentified sp. F44	- [a]	3.3	0.7	0.2
Unidentified sp. F45	GU067747	3.3	0.7	0.2
Unidentified sp. F75	- [a]	33.3	9.3	3.3
Unidentified sp. F76	- [a]	51.6	16.7	5.8
All ascomycetes and deuteromycetes		100.0	87.3	61.3
Zygomycetes				
Mortierella isabellina	- [a]	16.7	3.7	1.3
Mortierella ramanniana	- [a]	21.7	5.3	1.9
Mucor sp.	- [a]	23.3	4.7	1.6
All zygomycetes		50.0	11.0	4.9

[a] Identification based on mycelial morphology

one at GenBank, and one at the Department of Forest Mycology & Plant Pathology, Swedish University of Agricultural Sciences. The ITS sequence homology for delimiting fungal taxon was set at 98-100 %, and for delimiting at genus level, at 94-97 %. Internal Transcribed Spacer (ITS) sequences of each sequenced mycelial morphotype were deposited in GenBank (Table 1).

Data analysis

The aim of statistical analyses was to check what sampling effort (no. of samples taken / sampling attempts) would be required to reflect representative diversity of fungi inhabiting stumps. Species richness was analysed by calculating species accumulation curves (SACs) (Colwell and Coddington 1994): i) first, showing the relationship between number of samples taken and cumulative number of species detected in the clear-cut as a whole, ii) number of sampling attempts per stump and cumulative number of species detected after each five rounds of sampling, and iii) the relationship between number of samples taken and number of species detected in respect of stump diameter. In order to allow latter analysis, the stumps were separated in four arbitrary diameter classes: 11-20 cm (18 stumps), 21-30 cm (9), 31-40 cm (18) and 41-60 cm (15). SACs were calculated and the Figures drawn using R computer language (Ihaka and Gentleman 1996). Fungal community structures detected after each respective sampling round were pairwise compared in all combinations by calculating qualitative (S_S) and quantitative (S_N) Sorensen similarity indices (Magurran 1988). Similarly, we compared fungal communities detected in different stump diameter classes.

Results

Pure cultures of fungi were isolated from each of 300 wood samples taken. In total, 839 of fungal strains were obtained, meaning almost 3 strains per sample and 14 per stump on average. They represented 51 distinct mycelial morphotypes, 38 of which were identified to species level, 8 to genus level and 5 remained unidentified. Of them, 16 spp. belonged to basidiomycetes, 32 spp. to ascomycetes and deuteromycetes, and 3 spp. to zygomycetes (Table 1). Among basidiomycetes, *Sistotrema brinkmannii* was the most common fungus, detected in 83.3 % of stumps, followed by *Stereum sanguinolentum* (46.7 %) and *Phlebiopsis gigantea* (23.3 %). Among ascomycetes/deuteromycetes, the most common species was *Hormonema dermatioides* (58.3 %), followed by *Nectria fuckeliana* (56.7 %) and bark-beetle associated fungus *Ophiostoma piceae* (51.7 %). Bark-beetle associates representing the genus *Grossmania* were detected in 35.0 % of examined stumps. Root rot fungus *Heterobasidion parviporum* was isolated (only) from one sample (Table 1).

The used sampling effort (300 samples from 60 stumps) has to a large extent reflected species richness of (cultivable) fungi in stumps in the investigated clear-cut (Figure 1). Species accumulation curve raised rather sharp up to first 60 samples and flattened considerably after 120 samples. Figure 2 demonstrates that taking four samples (and subsequent isolations) could be enough to reasonably reveal species richness of (cultivable) fungi in a cut *P. abies* stump. Thus, the first round of sampling yielded 32 distinct species (in 60 stumps), the second increased detected diversity by 9, third by 5, fourth by 4, and fifth only by 1. The difference of proportions of species numbers detected during 4th and 5th samplings was statistically insignificant (chi-squared test, $p = 0.9$). There was no correlation between stump diameter and a number of species detected. Provided equal sampling effort, e.g. 45 samples from 9 stumps, this has revealed 30 distinct spp. in smallest, 11-20 cm diameter stumps, 32 spp. in 21-30 cm stumps, 34 spp. in 31-40 cm stumps, and again 30 spp. in largest 41-60 cm stumps (Figure 3).

Sorensen species similarity indices between different sampling rounds varied from moderately high to

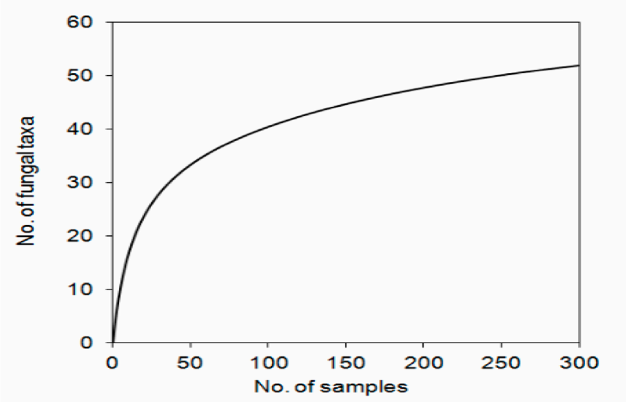

Figure 1. Richness of detected fungal taxa in relation to the number of samples taken on investigated clear-cut

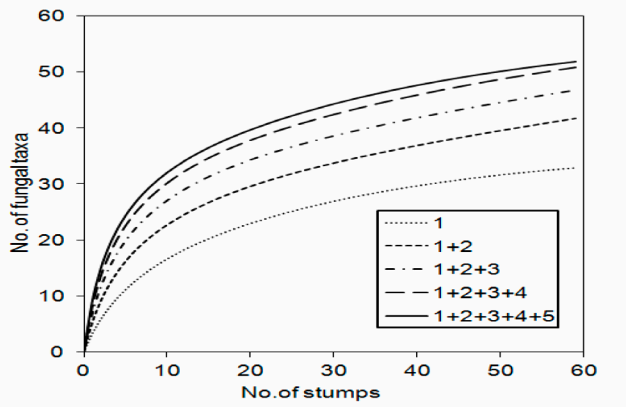

Figure 2. Accumulation curves of fungal taxa in stumps in relation to the number of samples taken from a stump (boxed)

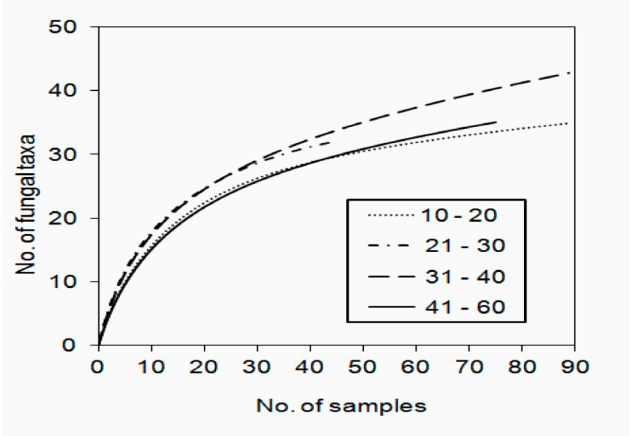

Figure 3. Richness of detected fungal taxa in relation to the number of samples taken and stump diameter class (boxed, cm)

high (S_S = 0.71-0.83 and S_N = 0.68-0.82), showing that mainly the same fungi have been isolated by each round. Sorensen species similarity indices between fungal communities detected in stumps of different diameter classes were also moderate to high (S_S = 0.75-0.81 and S_N = 0.63-0.71), demonstrating that *P. abies* stumps of different size (11 to 60 cm diameter, but felled trees were of about same age) on harvested forest sites are inhabited principally by the same fungi.

Discussion

Results of the present study indicate that used sampling effort to significant extent reflected species richness of cultivable fungi in *P. abies* stumps. This has certain implications when planning related fungal biodiversity studies in other types of CWD, e.g. logs or snags, especially when more expensive and laborious methods are to be applied, as e.g. direct molecular analyses from woody tissue. Yet, as compared with logs and snags, cut stumps are CWD of relatively small dimensions, but, on the other hand, they represent initially rather sparsely colonized (except for presence of certain endophytes, as e.g. *Ascocoryne* spp.) substrate that straightforward becomes accessible for colonization by airborne fungal spores through freshly cut and exposed wood. Consequently, the results of the study show that logging stumps harbor high species richness and diversity of mycelia of wood-inhabiting fungi.

Moreover, results of this work provided additional evidence that cut stumps are being inhabited by certainly specific community of wood-inhabiting fungi: each detected basidiomycete and most of asco- and deuteromycetes have been previously isolated from (or their sporocarps observed on) cut tree stumps during preceding studies (Vasiliauskas et al. 2002, 2004, 2005, Arhipova et al. 2011). Apparently, those fungi have fitness to successfully colonize freshly cut wood. In this respect, the following questions arise:

i) to which extent they represent fungal community of natural forest, where man-made freshly cut wood is absent, thus whether any of them represents rare, threatened or vulnerable species?

ii) to which extent the stumps are relevant for completion of the life cycle of those fungi (production of sporocarps and spore dispersal)?

Extensive sporocarp surveys demonstrate that, e.g. basidiomycetes detected during the present and related studies (Vasiliauskas et al. 2002, 2004, 2005, Arhipova et al. 2011) are typically widely distributed species commonly colonizing cut stumps. Normally, they colonize and regularly produce sporocarps also on other types of substrate, as newly dead (small dimension) tree stems and logs, fallen branches and living wounded trees that are abundant in managed forests of Europe (Eriksson et al. 1984, Ryvarden and Gilbertson 1994, Olofsson 1996, Vasiliauskas 1998a). Therefore, overall common occurrence of those fungi is not surprising.

Moreover, fungi colonizing cut stumps often include major tree pathogens. In regard to species detected during the present work, those are *Stereum sanguinolentum* (2nd most commonly detected basidiomycete) and *Heterobasidion parviporum*. The first is the most common cause up to 4-5 m long wound heart rot of *Picea* and *Abies* (Vasiliauskas 2001, Vasaitis 2013), while the second is root rot fungus and is the most economically important forest pathogen in Northern Hemisphere (Woodward et al. 1998). Rare occurrence of the latter on the studied plot could be explained by the fact that the site was initially healthy and that the felling was done in November, when spore dispersal is minimal. *Sistotrema brinkmannii*, in this work the most commonly isolated basidiomycete, is also known as opportunistic and occasional colonizer of mechanical stem wounds, that may also cause heart rot in living trees, although it is mostly known as cosmopolitan predominantly saprotrophic fungus (Vasiliauskas 1998b). *Neonectria fuckeliana*, the second most commonly isolated ascomycete, while being weak tree pathogen in Northern Hemisphere, proved to be invasive on *Pinus* in New Zealand, causing flute canker of significant impact (Crane et al. 2009). The ophiostomoid fungi *Ophiostoma piceae* and *Grossmania* spp., detected in every stump, are known as associates of numerous bark beetles that attack living trees (including the major insect pest *Ips typographus*), causing blue stain (Kirisits 2004). Finally, *Armillaria* spp. (but not isolated in this work), which is the second most important tree root rot fungus, is also known to commonly occur and produce sporocarps on stumps (Shaw and Kile 1991).

It, therefore, becomes evident that logging stumps, instead of being harbors for fungal biodiversity, in fact

represent the reservoirs for establishment, reproduction and further spread for number of common forest pathogens. On the other hand, as the stumps studied during the present study were rather "fresh" (cut 4 years ago), one might expect that perhaps "in the long run" they nevertheless will become valuable for sustaining biodiversity of vulnerable and threatened fungi. In this respect, relevant information is provided by extensive polypore sporocarp inventory carried out in old spruce forests of Finland, where a total of 14 252 cut stumps in 16 forest stands (managed and natural old-growth) have been surveyed (Penttilä et al. 2004). Based on the results of this study, Penttilä (2004) has made the following conclusions: i) not a single threatened species was found on cut stumps or on thin logging residues, ii) threatened species were almost entirely found in old-growth forests and on large-diameter logs in advanced stage of decay, and iii) *Heterobasidion*, a serious root rot pathogen of living spruce causing high economic losses in managed forests was found almost exclusively in cut stumps in managed stands. Similar conclusions were presented in the subsequent work (Berglund et al. 2011).

Present work, and a number of cited related studies, allows us to draw the following conclusions: i) among detected, all were common wood-inhabiting fungi typically producing sporocarps on several other types of dead wood than cut stumps ; ii) cut stumps commonly and regularly harbor important tree pathogens, representing bases for their establishment, reproduction and further spread; iii) no wood-inhabiting fungus of conservation interest was found in this study; iv) stump harvesting does not appear to pose any threat for fungi of conservation interest. Indeed, the last conclusion (iv) might appear too strong for such rather fragmented study, but it is also based on numerous cited works. It is also in good agreement with our recently conducted investigations, where fungal communities in cut spruce stumps were examined using direct molecular analysis of wood samples collected from 41 clear-cuts at seven localities along a latitudinal gradient from northern to southern Sweden. A total of 1335 Operational Taxonomic Units (OTUs, or presumed species) were detected and none of them represented threatened species (Kubart et.al 2016). Moreover, it might be added here that cut stumps of spruce serve ecological niche for reproduction for one of the most economically important insect pests, namely *Hylobius abietis* (Viri 2004).

Drawn conclusions are therefore different from those made by Toivanen et al. (2012), where it was pointed out that stump removal / harvesting in time perspective can cause decline of stump-living fungi. Such reservation is likely improbable, because stump harvesting is to be accomplished in a fraction of clear-cuts, and from thinned stands the stumps will never be removed.

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