Fungal Ecology 54 (2021) 101106

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Deadwood substrate and species-species interactions determine the release of volatile organic compounds by wood-decaying fungi

M. Mäki [a,b,*], T. Mali [c], H. Hellén [d], J. Heinonsalo [a,b,c,d], T. Lundell [c], J. Bäck [a,b]

[a] Institute for Atmospheric and Earth System Research (INAR) / Forest Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland
[b] Department of Forest Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland
[c] Department of Microbiology, Faculty of Agriculture and Forestry, University of Helsinki, Finland
[d] Finnish Meteorological Institute, Helsinki, Finland

ARTICLE INFO

Corresponding Editor: Peter H.W. Biedermann

Keywords:
Volatile organic compound
Decomposition
Wood decay
Basidiomycota
Fungal interactions
White rot
Brown rot
Enzyme activity
Coniferous wood
Carbon cycling

ABSTRACT

Wood-decaying fungi in the phylum Basidiomycota play a significant role in the global carbon cycle, as they decompose deadwood effectively. Fungi may compete for utilizable substrate and growth space by producing soluble metabolites and by releasing volatile organic compounds (VOCs). We determined the role of wood substrate (Scots pine or Norway spruce) on the generation of hyphal biomass, secreted metabolites and enzyme activities, wood decomposition rate, and fungal species-species interactions on VOC release. We studied one brown-rot species (*Fomitopsis pinicola*) and two white-rot species (*Phlebia radiata* and *Trichaptum abietinum*) cultivated individually or in combinations. Wood substrate quality influences VOC release by the wood-decaying fungi, with signature differences caused by the decomposition trait (brown rot or white rot) and species-species interactions. VOC release was higher in the cultures of Basidiomycota than in uncolonized sawdust. Fungal biomass, decomposition activity, iron reduction, enzyme activities, oxalate anion content, and oxalic acid production explained VOC release from decaying wood.

1. Introduction

Wood-decomposing fungi in the phylum Basidiomycota, such as species causing white and brown rot, are a crucial element in the global carbon cycle (Eastwood et al. 2011; Floudas et al. 2012). During the active decomposition phase, deadwood and forest litter are colonized by a multitude of saprotrophic fungal species belonging to Basidiomycota and Ascomycota (Lundell et al. 2014). Ascomycota are the most dominant fungi during the decay stages of deadwood in boreal forests (Rajala et al., 2012), most likely due to scarcity of available soil nitrogen, while Basidiomycota remain the most abundant group of fungi throughout long-term decomposition processes in European forest deadwood (Hoppe et al., 2016). Decomposition rates and chemical conditions in deadwood vary during the decomposition process (Bani et al. 2018). Basidiomycetous brown-rot fungi mainly decompose wood polysaccharides (cellulose and hemicelluloses), while white-rot fungi decompose all wood components including lignin and cellulose (Eastwood et al. 2011; Floudas et al. 2012; Lundell et al. 2014; Kuuskeri et al. 2016).

Volatile organic compounds (VOCs) are important in chemical interactions between bacteria, fungi, and plants (Schulz-Bohm et al. 2017). Earlier studies have indicated that fungal and microbial decomposers may produce and release VOCs e.g., to secure their growth space and resource availability (Müller et al. 2013; Boddy 2016; El Ariebi et al. 2016; Hiscox et al. 2018). Competition between species is the main form of interaction among filamentous fungi capable of decaying wood (Boddy 2000; Hiscox et al. 2018). The release of VOCs may enhance the mycelial growth of interacting partners (Evans et al. 2008), increase microbial activity in the soil (Adamczyk et al. 2015), and support beneficial interactions between fungal species (Schulz-Bohm et al. 2017). The generation and release of VOCs by wood-decaying fungi is dependent on species and interaction combinations (Evans et al. 2008; Mali et al. 2019).

Fungal hyphae and fruit bodies have the potential to synthesize and emit volatile isoprenoids, such as monoterpenoids and sesquiterpenes, and oxygenated VOCs (C_4-C_{15} alcohols, ketones, and acetates) (Ewen et al. 2004; El Ariebi et al. 2016). VOCs, such as sesquiterpenes and monoterpenoids, typically play different ecological roles in interactions

* Corresponding author. Institute for Atmospheric and Earth System Research, Finland.
E-mail address: mari.maki@helsinki.fi (M. Mäki).

https://doi.org/10.1016/j.funeco.2021.101106
Received 23 November 2020; Received in revised form 3 August 2021; Accepted 6 August 2021
Available online 22 September 2021

between organismal groups. Nitrogen availability for plants may change through VOC-induced alteration of microbial nitrification and mineralization activities in soil, carbon cycling may be affected by changes in organic matter decomposition and microbial activities, and plant growth together with host recognition by mycorrhizal fungi may be enhanced (Smolander et al. 2012; Ditengou et al. 2015; Adamczyk et al. 2018; Schenkel et al. 2018). Oxygenated VOCs (oVOCs) are water-soluble and many oVOCs may also be oxygenated degradation products of larger organic molecules synthesized by plants. The diversity of organic volatile compounds is huge, and isoprenoids are universal secondary metabolites of plants.

Decomposers in deadwood and litter are regulated by environmental conditions and substrate quality (Bani et al. 2018). Substrate quality (anatomical structure and chemical composition of volatile and non-volatile organic compounds in wood) may have a major role in fungal-promoted release of VOCs. It has been reported that monoterpene release from decomposing *Pinus sylvestris* litter was five to ten times higher than from decomposing *Picea abies* litter at an early stage of decomposition (Isidorov et al. 2010). The warming climate may alter microbial communities and shift ecological interactions between plants and soil microbes (Classen et al. 2015). Microbial processes may impact atmospheric chemistry, as the boreal forest floor may contribute significantly to ecosystem emissions of monoterpenes in early spring and autumn (Mäki et al. 2019a). Sesquiterpene release from microbial processes is especially important, as sesquiterpenes have a major role in oxidation reactions occurring under the tree canopy (Hellén et al. 2018).

This study aimed to determine the effect of woody substrates, either *Pinus sylvestris* (Scots pine) or *Picea abies* (Norway spruce), on fungal VOC synthesis and release, on the growth and metabolic activities of three wood-decaying Basidiomycota (class Agaricomycetes) species with different decomposition strategies, namely *Fomitopsis pinicola* (a brown-rot fungus), and *Phlebia radiata* and *Trichaptum abietinum* (white-rot fungi). These fungal species were chosen as they are representatives of the orders *Polyporales* and *Hymenochaetales*, and common deadwood saprotrophs in boreal and temperate forests (Kuuskeri et al. 2015; Mali et al. 2017; Shah et al. 2018). Moreover, the three fungal species and their combinations were studied previously on agar and liquid media (Mali et al. 2017), which supported experimental planning of this research. We carried out these concurrent solid-state cultivations in wood, as fungal hyphal interactions may markedly differ on laboratory agar media compared to experiments using natural substrates (Hiscox and Boddy 2017; Mali et al. 2017).

The main aim of this study was to determine the role of wood substrate – either Scots pine or Norway spruce – on the generation of hyphal biomass, secreted metabolic activities, wood decomposition, and fungal species-species interactions in relation to VOC release. This study complements our previous findings concerning signature VOCs released by the same fungi growing in Norway spruce (Mali et al. 2019). In the current study, we hypothesized that:

1. Interactions of brown-rot and white-rot fungi accelerate VOC release compared to single-species cultivations.
2. VOC release from wood by brown rot is more substantial due to the more rapid hyphal growth and efficient decomposition of coniferous wood compared to white-rot fungi.
3. VOC synthesis and release by wood-decaying fungi depends on their growth substrate (either spruce or pine wood).
4. Fungal metabolism indicators, such as hyphal ergosterol content, enzyme activities (laccase, β-glucosidase, chitinase, and acidic peptidase), oxalic acid production, and iron reduction explain the VOC release of wood-decaying fungi.

2. Material and methods

2.1. Experimental set-up

Three wood-decaying fungi of Basidiomycota (*Fomitopsis pinicola* isolate FBCC1181 Fp, *Phlebia radiata* isolate FBCC0043 Pr, *Trichaptum abietinum* FBCC0110 Ta) were combined using wood substrate cultivations, similar to our previous study (Mali et al. 2019). The fungal isolates are stored in the FBCC sub-collection of the Microbial Domain Culture Collection HAMBI, Helsinki Institute of Life Sciences, University of Helsinki. The fungal single-species (Fp, Ta or Pr) and species combinations (FpPr, FpTa, FpPrTa) were cultivated in three parallel cultures on

Table 1
The concentrations of isoprene, monoterpenoids, sesquiterpenes, and oxygenated volatile organic compounds (oVOCs) were determined based on the detection limit of each compound. VOCs marked with * were calibrated using other sesquiterpenes (isolongifolene, longicyclene and ß-caryophyllene) as standards. All other VOCs were calibrated using authentic standards. One sesquiterpene was only tentatively identified and was therefore marked as SQT1*.

Compound	Concentration (μg m^{-3})	Compound	Concentration (μg m^{-3})	Compound	Concentration (μg m^{-3})
isoprene	0.040	**Sesquiterpenes**		**oVOCs**	
Monoterpenoids		longicyclene	0.012	Geraniol	0.055
α-pinene	0.131	isolongifolene	0.007	α-bisobolol	0.436
camphene	0.002	ß-caryophyllene	0.008	Verbenone	0.698
Δ-3-carene	0.036	aromadendrene*	0.006	Nuciferol	9.549
ß-pinene	0.003	α-gurjunene	0.940	methyl-3-furoate	2.738
myrcene	0.009	α-humulene	0.001	1-butanol	0.445
1,8-cineol	0.0005	ß-farnesene	0.061	Isopropanol	0.006
linalool	0.232	isocaryophyllene*	0.046	2-butanone	0.018
limonene	0.056	SQT1*	0.211	1-penten-3-ol	0.002
p-cymene	0.001	α-buinesene*	0.051	1-pentanol	0.046
terpinolene	0.002	γ-muurolene*	0.061	3-methyl-2-buten-1-ol	0.031
bornylacetate	0.012	α-bisabolene*	0.044	butyl acetate	0.025
		β-himachalene*	0.009	cis-3-hexen-1-ol	0.043
		α-muurolene*	0.008	trans-3-hexen-1-ol	0.043
		Δ-cadinene*	0.046	trans-2-hexen-1-ol	0.043
		α-curcumene	0.026	1-hexanol	0.014
				cis-2-hexen-1-ol	0.008
				1-octen-3-ol	0.009
				6-methyl-5-heptene-2-one	0.700
				cis-3-hexenyl acetate	0.058
				hexyl acetate	0.005
				trans-2-Hexenyl-acetate	0.004
				α-pinenepxide	0.0003
				3-octanone	1.334
				methyl-12-furoate	0.119

either Norway spruce or Scots pine sawdust. Ten grams (dry weight) of wood sawdust were dry-autoclaved (121 °C, 15 min) in 250-ml glass Erlenmeyer flasks before inoculating the cultures with a piece of mycelium on malt-extract agar. The culture flasks with closed with cellulose stoppers. The cultures were incubated at room temperature (21–23 °C) for 12 weeks, and their moisture level was kept constant by adding sterile Milli-Q water (Mali et al. 2019). The effect of fungal interactions on VOC release was determined in a factorial design by growing fungal species in several combinations: either as individual fungus isolates, or in pairs, or all three on the same substrate. Each combination included four biological replicates as four individual culture flasks (n = 4).

2.2. Analytical methods

2.2.1. VOC flux measurements

We measured the VOC release of each fungal combination at room temperature on three occasions: 4, 8, and 12 weeks after inoculation of the fungi (Mali et al. 2019). The flasks were flushed with VOC and oxidant-free air for 10–15 min before sampling. VOCs were sampled using Tenax TA-Carboback-B adsorbent tubes by sucking air from the closed headspace for 1.5 h (Fig. S1). Both ingoing and outgoing air were sampled, and the flux was calculated from the concentration difference between ingoing and outgoing air, as described in Mäki et al. (2017; 2019c). Plain sawdust was measured as a control with four replicates. Ten glass flasks were measured during one measurement cycle and two replicates of a certain fungal culture or plain sawdust were measured in one measurement cycle. First, we measured uninoculated spruce sawdust and fungal cultures on spruce sawdust, where species were growing either individually or in combination of all three together using two measurement cycles. Second, we measured the fungal cultures on spruce sawdust, where species were growing in pairs. Third, we measured plain pine sawdust and fungal cultures on pine sawdust, where species were growing individually. Finally, we measured fungal cultures on pine sawdust, where species were growing in pairs or all three together.

The concentrations of isoprene, monoterpenoids, sesquiterpenes, and different oVOCs were determined from the adsorbent tubes (Mäki et al. 2019c) (Table 1). The adsorbent tubes were analysed within 2 weeks using a thermal desorption (PerkinElmer TurboMatrix 650;

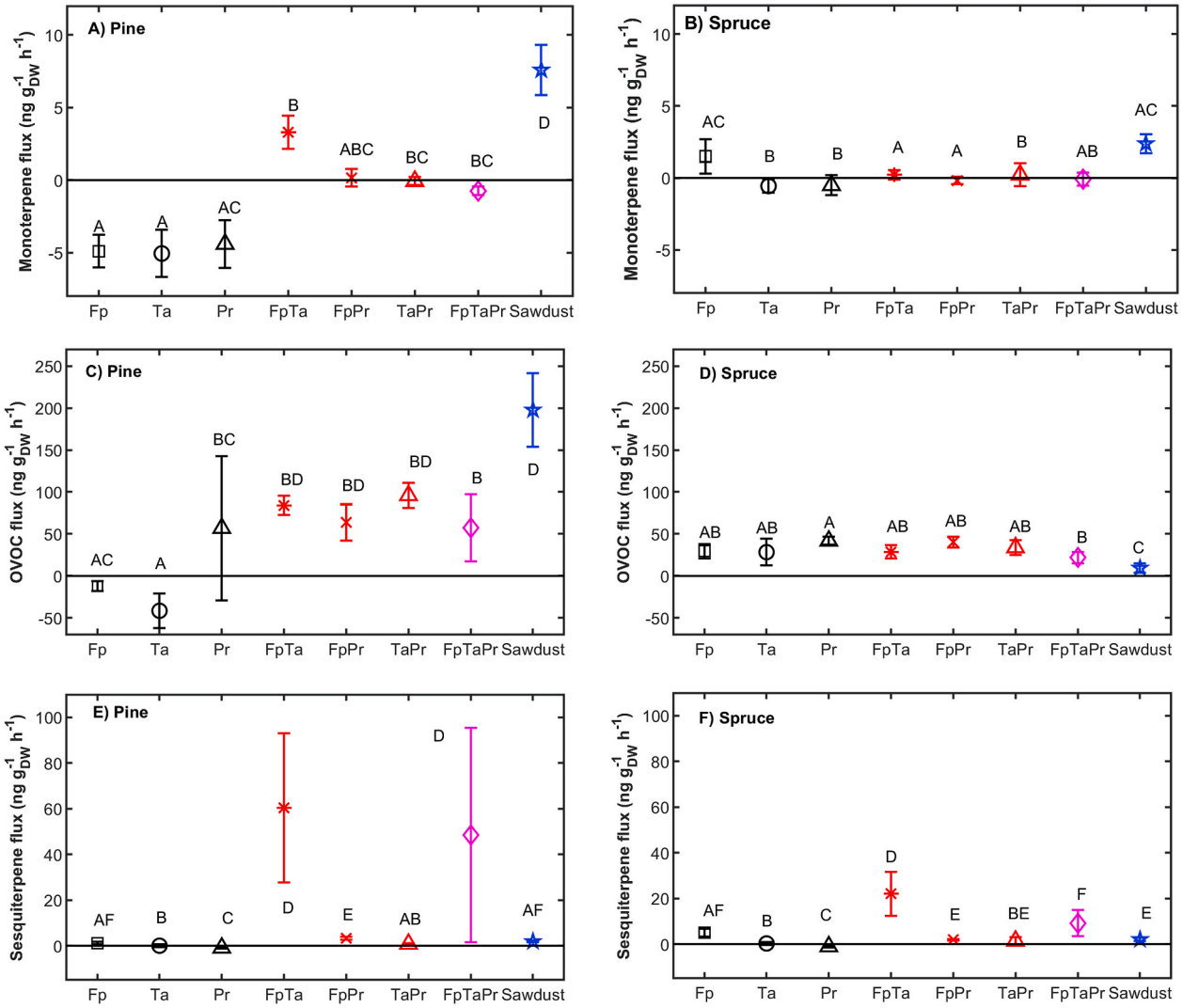

Fig. 1. Release of (A-B) total monoterpenoids, (C–D) oxygenated volatile organic compounds (oVOCs), and (E–F) total sesquiterpenes by fungal cultures growing on pine and spruce sawdust. The different cultures consist of fungal species growing individually (Fp = *Fomitopsis pinicola,* Ta = *Trichaptum abietinum*, and Pr=*Phlebia radiata*) and in combinations. The flux rates with standard deviations are means of four biological replicates, and the flux rate of each replicate is the mean of three measurement times. Letters (A–F) indicate a statistically significant difference between fungal cultures ($p < 0.1$°).

PerkinElmer, Waltham, MA, USA) –gas chromatograph (PerkinElmer Clarus 600) –mass spectrometer (PerkinElmer Clarus 600T) (Aaltonen et al. 2011). The absence of authentic standards for α-buinesene, γ-muurolene, α-bisabolene, β-himachalene, α-muurolene, and Δ-cadinene may increase the uncertainty of VOC quantification. The adsorbent tubes were analysed by desorbing VOCs from the tubes into a helium flow using thermal desorption (300 °C), by cryofocusing VOCs in the cold trap (−30 °C), by injecting VOCs into a gas chromatograph column using rapid heating (300 °C), and by detecting them with a mass spectrometer. The instrument was calibrated by measuring four known concentrations of different VOCs in standard mixtures in methanol solutions by injecting (5 μL) standards into the sample tubes. The VOCs were identified by matching their retention times and mass spectra to the authentic standards. Sesquiterpenes without authentic standards were only tentatively identified by comparing them to the NIST (the National Institute of Standards and Technology) mass spectral library and calibrated using other sesquiterpenes (Mäki et al. 2019c). The detection limit for VOC quantification was 0.0003–9.549 μg m^{-} depending on the compound, calculations were based on Mäki et al. (2017) (Table 1).

The fungal VOC flux (VOC_F, ng $g_{DW}{}^{-1}$ h^{-1}) of each compound from each fungal culture was determined using equation (1) below. We calculated the difference between ingoing (C_{IN}, ng h^{-1}) and outgoing (C_{OUT}, ng h^{-1}) samples, divided that with the dry weight of the wood sawdust and fungal biomass (m_{DW}, g) in each flask, and subtracted the mean VOC release of the uncolonized sawdust (VOC_s, ng $g_{DW}{}^{-1}$ h^{-1}).

$$VOC_F = \frac{C_{OUT} - C_{IN}}{m_{DW}} - VOC_s \quad (1)$$

2.2.2. Fungal enzyme activities, metabolites, and wood decomposition

To characterize fungal metabolic activities, we analysed secreted carbohydrate-active enzyme (CAZYme) activities and metabolic products from the liquid phase of the fungal cultures after each VOC sampling (4, 8, and 12 week after cultivation began). Fungal hyphal growth as generation of biomass in the solid wood sawdust susbstrates was determined by the ergosterol content (Shah et al. 2018; Mali et al. 2019). Decomposition activity was determined by calculating the loss of wood mass as decrease in dry weight of the culture solids (Mali et al. 2019). We calculated the mass loss of spruce and pine wood at the same three time points (after 4, 8 and 12 weeks of cultivation). β-glucosidase activity was defined using 4-nitrophenyl β-D-glucopyranoside as the enzyme substrate (Rytioja et al. 2014), and laccase and manganese peroxidase (MnP) activities using ABTS and Mn^{2+} ions as substrates, respectively (Kuuskeri et al. 2015). Iron reduction, chitinase and acidic peptidase activities, and chelated oxalate anion (oxalate) content were analysed using previously described analytical methods (Shah et al. 2018). Oxalic acid production was determined by HPLC analysis (Mattila et al. 2017).

2.3. Statistical analyses

In this study, we used a factorial laboratory experiment design by cultivating the fungal species either individually, in pairs, or all three in combination on the same substrate, i.e. either on spruce or pine sawdust. Each species combination included four biological replicate cultures (Mali et al. 2019). As the data were not normally distributed, we used the non-parametric Kruskal-Wallis test (n = 4, df = 1, significance level of $p < 0.100^{\circ}$) to determine whether the total monoterpene, sesquiterpene, and oVOC fluxes of the fungal combinations differed statistically from each other at a given sampling time on both wood substrates (Fig. 1).

We used linear mixed-effects models to explain the effect on VOC release ($Flux_{VOC}$) caused by the fungal species combinations, extracellular enzyme activities (chitinase, β-glucosidase, laccase, manganese peroxidase, and acidic peptidase), iron reduction capacity, generation of fungal biomass (ergosterol accumulation), chelated oxalate anion content, and production of oxalic acid (Tables 2 and 3). We tested the effect of a fixed intercept parameter (B_0), fungal species combination (B_F), chitinase activity (B_C), β-glucosidase activity (B_β), acidic peptidase activity (B_P), iron reduction capacity (B_R), ergosterol accumulation (B_E), production of oxalic acid (B_{OA}), laccase activity (B_L), manganese peroxidase (MnP) activity (B_{MnP}), mass loss (B_{ML}), and chelated oxalate anion content (B_{COA}). VOC release was tested using equation (2):

$$Flux_{VOC} = B_0 + B_F + B_C + B_\beta + B_P + B_R + B_E + B_{OA} + B_L + B_{MnP} + B_{ML} + B_{COA} + \in \quad (2)$$

The error term ∈ in equation (2) was formulated as:

Table 2
The linear mixed-effects model was used to explain the effect of fungal species combination (Fungi), ergosterol content, acidic peptidase activity, β-glucosidase activity, oxalic acid production, chitinase activity, iron reduction, laccase activity, manganese peroxidase (MnP) activity, wood mass loss, and chelated oxalate anion content (oxalate) on the release of monoterpenoids, sesquiterpenes, oxygenated volatile organic compounds (oVOCs), and individual compounds that were well explained by the fungal metabolic factors on spruce sawdust. The R^2 and p values indicate the significance of linear regression. N was 12 for all fungal cultures.

Sesquiterpenes			Monoterpenoids			oVOCs			Isoprene		
Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2
Intercept	0.26	0.71	Intercept	0.58	0.78	Intercept	<0.001***	0.64	Intercept	<0.001***	0.78
iron reduction	<0.01**		Oxalate	<0.05*		laccase	<0.05*		MnP	<0.01**	
			iron reduction	<0.05*		MnP	<0.001***		peptidase	<0.05*	
			mass loss	<0.1°		oxalate	<0.001***				
						peptidase	<0.001***				
β-pinene			**Δ-3-carene**			**β-himachalene**			**Δ-cadinene**		
Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2
Intercept	<0.1°	0.82	Intercept	0.13	0.80	Intercept	<0.001***	0.68	Intercept	<0.1 °	0.76
chitinase	<0.01**		laccase	<0.05*		β-glucosidase	<0.01**		oxalic acid	<0.001***	
iron reduction	<0.001***		oxalate	<0.01**		oxalic acid	<0.001***				
mass loss	<0.001***		oxalic acid	<0.01**							
			iron reduction	<0.001***							
			mass loss	<0.001***							
Iso-longifolene			**nuciferol**			**trans-3-hexen-1-ol**			**3-methyl-2-buten-1-ol**		
Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2
Intercept	0.63	0.72	Intercept	0.66	0.82	Intercept	<0.01**	0.69	Intercept	0.50	0.44
β-glucosidase	<0.05*		iron reduction	<0.01**		β-glucosidase	<0.01**		oxalate	<0.001***	
chitinase	<0.1°		mass loss	<0.001***		oxalate	<0.001***				
iron reduction	<0.001***										

Table 3
The linear mixed-effects model explained the effect of fungal growth combination (Fungi), ergosterol content, acidic peptidase activity, β-glucosidase activity, oxalic acid production, chitinase activity, iron reduction, laccase activity, and manganese peroxidase (MnP) activity, wood mass loss, and chelated oxalate anion content (oxalate) on the release of monoterpenoids, sesquiterpenes, oxygenated volatile organic compounds (oVOCs), and individual compounds, well explained by the fungal metabolic factors, on pine sawdust. The R^2 and p values show the significance of linear regression.

Sesquiterpenes			Monoterpenoids			oVOCs			Isoprene		
Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2
Intercept	<0.001***	0.74	Intercept	0.37	0.84	Intercept	0.67	0.59	Intercept	<0.05*	0.90
β-glucosidase	<0.05*		ergosterol	<0.05*		laccase	<0.001***		ergosterol	<0.001***	
oxalate	<0.001***		oxalate	<0.01**		oxalate	<0.001***		oxalate	<0.001***	
chitinase	<0.001***					oxalic acid	<0.001***		peptidase	<0.1°	
β-pinene			**limonene**			**Nuciferol**			**SQT1***		
Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2
Intercept	<0.01**	0.78	Intercept	0.53*	0.74	Intercept	<0.001***	0.86	Intercept	<0.01**	0.77
ergosterol	<0.001***		ergosterol	<0.05*		ergosterol	<0.001***		ergosterol	<0.001***	
oxalic acid	<0.05*					oxalic acid	<0.001***				
iron reduction	<0.01**					chitinase	<0.01**				
α-curcumene			**α-buinesene**			**α-bisabolene**			**butyl acetate**		
Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2	Fixed effects	p value	R^2
Intercept	<0.001***	0.75	Intercept	<0.001***	0.74	Intercept	<0.01**	0.74	Intercept	0.28	0.86
β-glucosidase	<0.01**		oxalate	<0.001***		ergosterol	<0.001***		ergosterol	<0.01**	
oxalate	<0.001***		chitinase	<0.001***		β-glucosidase	<0.001***		laccase	<0.01**	
chitinase	<0.001***								oxalate	<0.001***	
mass loss	<0.1°										

$$\in = \propto_{WF} + \propto_{RF} + e, \quad (3)$$

where $\propto_{WF}$ contains random parameters associated with the interaction of the measurement months (1–3) and fungal species combination, $\propto_{RF}$contains random parameters associated with the interaction of the number of biological replicates (1–4) and fungal species combination, and *e* was a random error term. If the effect of fungal species combination was insignificant on the release of certain compounds or compound groups, we added it to the model as an error parameter $\propto_F$ containing random parameters associated with the fungal species combination. The final model included only those parameters with a statistically significant effect on compound or compound group (monoterpenoids, sesquiterpenes, and oVOCs) with significance levels of $p < 0.100^{o}$, $p < 0.050$*, $p < 0.010$**, $p < 0.001$*** (Tables 2 and 3). The R^2 value indicates the explanatory power of the model when compared with VOC flux measurements (Tables 2 and 3). The linear mixed-effects models were determined separately on experiments performed on spruce and pine wood growth substrates. N was 12 for all fungal cultures.

Exponential and linear regression of the VOC fluxes with selected fungal enzyme activities (chitinase, β-glucosidase, acidic peptidase), iron reduction capacity, generation of fungal biomass (ergosterol accumulation), and production of oxalic acid were tested for each fungal combination. The R^2 and p values < 0.1° indicate the statistical significance of the exponential regression (Supplementary Tables 1 and 2).

3. Results

3.1. Spruce as a substrate

Spruce wood decomposition by brown-rot and white-rot species released oVOCs (21.9–41.8 ng g_{DW}^{-1} h^{-1}, std 4.75–15.94 ng g_{DW}^{-1} h^{-1}),

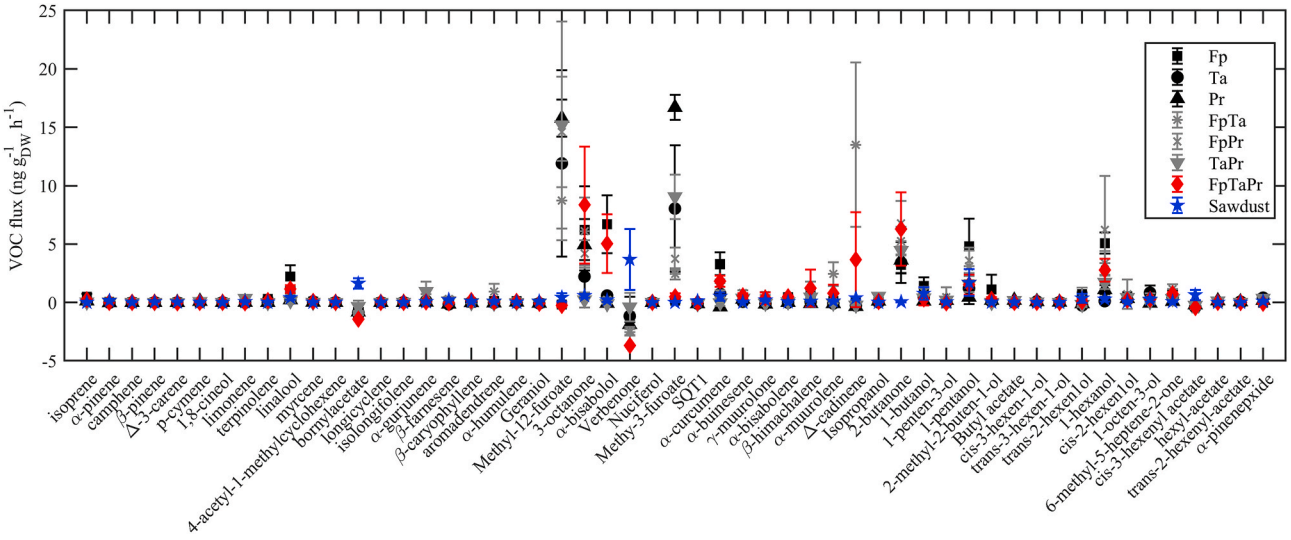

Fig. 2. Release of individual volatile organic compounds (VOCs) by fungal cultures growing individually (Fp = *Fomitopsis pinicola,* Ta = *Trichaptum abietinum*, and Pr=*Phlebia radiata*) and in combinations on spruce sawdust. The flux rates are calculated mean values with standard deviation of four biological replicates and the flux rate of each replicate is the mean value of the three measurement times (4, 8 and 12 weeks).

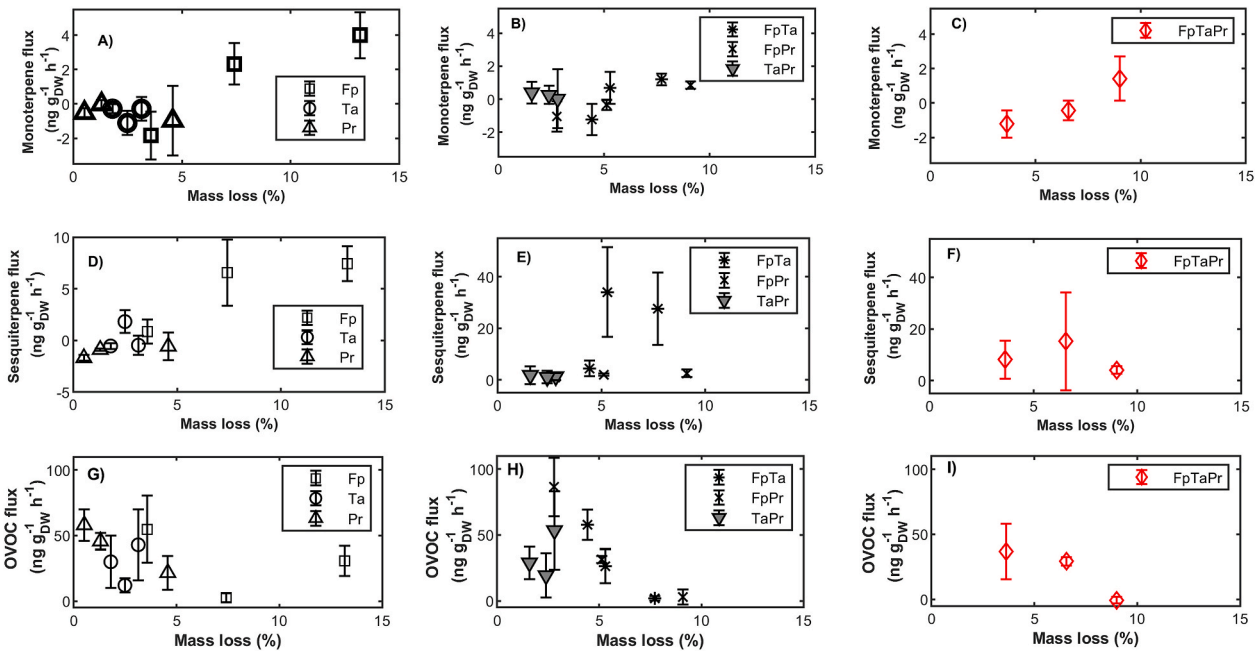

Fig. 3. The relationship between wood mass loss and release of monoterpenoids (A–C), sesquiterpenes (D–F), and oxygenated volatile organic compounds (oVOCs) (G–I) from the fungal cultures on spruce sawdust. Fungal species were grown individually (Fp = *Fomitopsis pinicola,* Ta = *Trichaptum abietinum*, and Pr=*Phlebia radiata*) and in combinations. The mean values with standard deviation show variation of the four biological replicates during the different cultivation weeks.

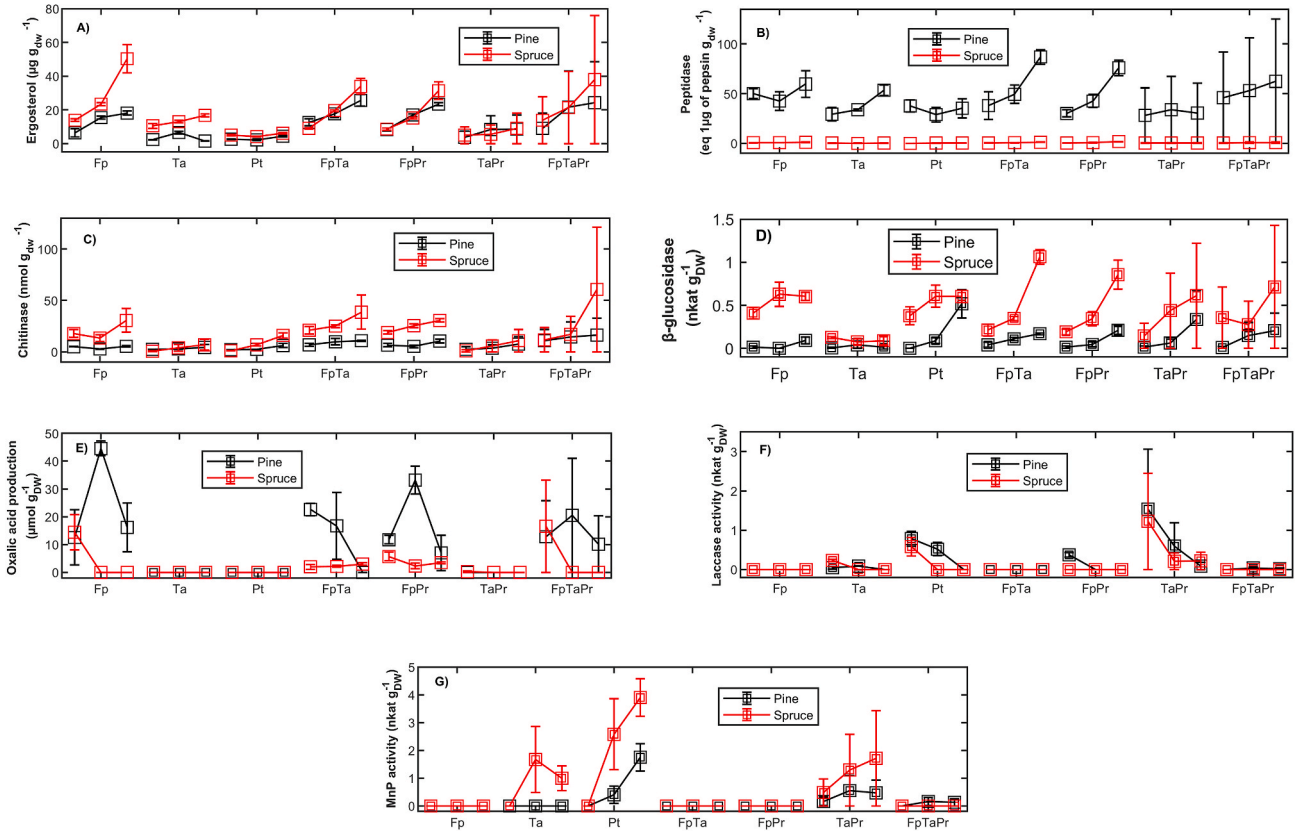

Fig. 4. (A) Ergosterol content, (B) acidic peptidase activity, (C) chitinase activity, (D) β-glucosidase activity, (E) oxalic acid production, (F) laccase activity and (G) manganese peroxidase (MnP) activity in fungal cultures on spruce and pine sawdust during cultivation weeks 4, 8 and 12. The mean values with standard deviations show variation of the four biological replicates. Mali et al. (2019) have published this data earlier.

sesquiterpenes (0.3–22.0 ng g_{DW}^{-1} h^{-1}, std 0.36–9.64 ng g_{DW}^{-1} h^{-1}), and a small amount of monoterpenoids (0.2–1.5 ng g_{DW}^{-1} h^{-1}, std 0.33–1.20 ng g_{DW}^{-1} h^{-1}) (Fig. 1). Spruce sawdust itself released oVOCs (9.4 ng g_{DW}^{-1} h^{-1}, std 5.80 ng g_{DW}^{-1} h^{-1}), sesquiterpenes (2.0 ng g_{DW}^{-1} h^{-1}, std 0.74 ng g_{DW}^{-1} h^{-1}), and monoterpenoids (2.4 ng g_{DW}^{-1} h^{-1}, std 0.66 ng g_{DW}^{-1} h^{-1}). Combinations of brown-rot and white-rot fungi increased sesquiterpene release compared to individual fungal species. The total sesquiterpene fluxes were highest with a combination of Fp and Ta ($p < 0.05$*), and in the co-cultivation of all three species ($p < 0.05$*). Monoterpene release was highest when brown-rot fungus Fp was growing individually compared to the two white-rot species ($p < 0.05$*). We observed hardly any differences in oVOC fluxes between the cultures of single species or different combinations of fungal species. We also observed indications of monoterpenoid uptake or bioconversion by Ta (−0.6 ng g_{DW}^{-1} h^{-1}) and Pr (−0.5 ng g_{DW}^{-1} h^{-1}) and sesquiterpene uptake or bioconversion by Pr (−1.0 ng g_{DW}^{-1} h^{-1}): the VOC fluxes from these species were smaller than the mean fluxes measured from uncolonized spruce wood sawdust without mycelia.

The fluxes were dominated by oVOCs, such as methyl-12-furoate, methyl-3-furoate, 1-hexanol, 3-octanone, 2-butanone, and α-bisabolol, and sesquiterpenes such as α-curcumene, α-muurolene, and Δ-cadinene (Fig. 2).

Increasing mass loss of spruce sawdust elevated the total monoterpenenoid release of brown-rot species Fp when it was growing both individually and together with the two-white rot species Ta and Pr (Fig. 3). Increasing mass loss was also found to accelerate sesquiterpene release by Fp but only in individual cultures. The opposite trend was observed for oVOC fluxes, which were highest when living fungal biomass was lowest, indicating that oVOCs were emitted at an early stage of fungal growth and monoterpenoids at later stages, during the active period of interaction and fungal biomass growth into spruce substrate (Fig. 3).

Enzyme activities (acidic peptidase, chitinase, and β-glucosidase) and generation of fungal biomass (ergosterol content) increased in the fungal cultures during cultivation on spruce sawdust, while oxalic acid production was highest at the beginning of the experiment in individual cultures of the brown-rot fungus Fp and in the three-species co-cultivation (Fig. 4). Enzyme activities and fungal biomass were highest in the single-species cultures of Fp compared to the two white-rot fungi (Ta and Pr) individually and in their paired cultures (Fig. 4). Enzyme activities and generation of fungal biomass showed an increasing tendency in combinatory cultures including the brown-rot species (combinations FpTa, FpPr, and FpTaPr).

3.2. Pine as a substrate

Pine wood decomposition released oVOCs (57.0–96.0 ng g_{DW}^{-1} h^{-1}, std 11.37–85.95 ng g_{DW}^{-1} h^{-1}), sesquiterpenes (0.8–60.3 ng g_{DW}^{-1} h^{-1}, std 0.24–46.98 ng g_{DW}^{-1} h^{-1}), and monoterpenoids (0.2–3.3 ng g_{DW}^{-1} h^{-1}, std 0.60–1.13 ng g_{DW}^{-1} h^{-1}) (Fig. 1). Pine sawdust itself released oVOCs (197.6 ng g_{DW}^{-1} h^{-1}, std 43.63 ng g_{DW}^{-1} h^{-1}), sesquiterpenes (1.8 ng g_{DW}^{-1} h^{-1}, std 0.27 ng g_{DW}^{-1} h^{-1}), and monoterpenoids (7.6 ng g_{DW}^{-1} h^{-1}, std 1.73 ng g_{DW}^{-1} h^{-1}). We observed a decrease in monoterpeneoid fluxes caused by all species (Fp: 4.9 ng g_{DW}^{-1} h^{-1}, Pr: 4.4 ng g_{DW}^{-1} h^{-1} and Ta: 5.1 ng g_{DW}^{-1} h^{-1}) and a decrease in oVOC fluxes by Fp and Ta (−12.1 and −41.5 ng g_{DW}^{-1} h^{-1}). The total sesquiterpene fluxes were highest in FpTa co-cultivations ($p < 0.001$***) or when all three species were growing together ($p < 0.01$**). Interaction between Fp and Ta accelerated the VOC release of monoterpenoids on pine sawdust, while the trend was less clear on spruce sawdust (Fig. 1). In total, fungal VOC release was higher on pine wood substrate compared to spruce wood substrate.

The most common VOCs released by the fungal cultures on pine wood were sesquiterpenes, such as α-muurolene, and Δ-cadinene, and oVOCs such as methyl-12-furoate, 3-octanone, 2-butanone, and 1-hexanol (Fig. 5).

Unlike on spruce sawdust, increasing decomposition rate of pine sawdust did not explain VOC release by the fungal cultures (Fig. 6). Monoterpenoid uptake or bioconversion by individual fungal cultures was lower when

the decomposition rate of pine sawdust was higher. Sesquiterpene release increased with a higher decomposition rate, when all three species were growing together. The negative values of wood mass loss are most likely measurement errors.

Hyphal biomass (ergosterol content) was highest for brown-rot fungus Fp compared to the two white-rot fungi (Ta, Pr) on pine sawdust (Fig. 4), but differences in enzyme activities (acidic peptidase and chitinase) between the cultures were less apparent compared to

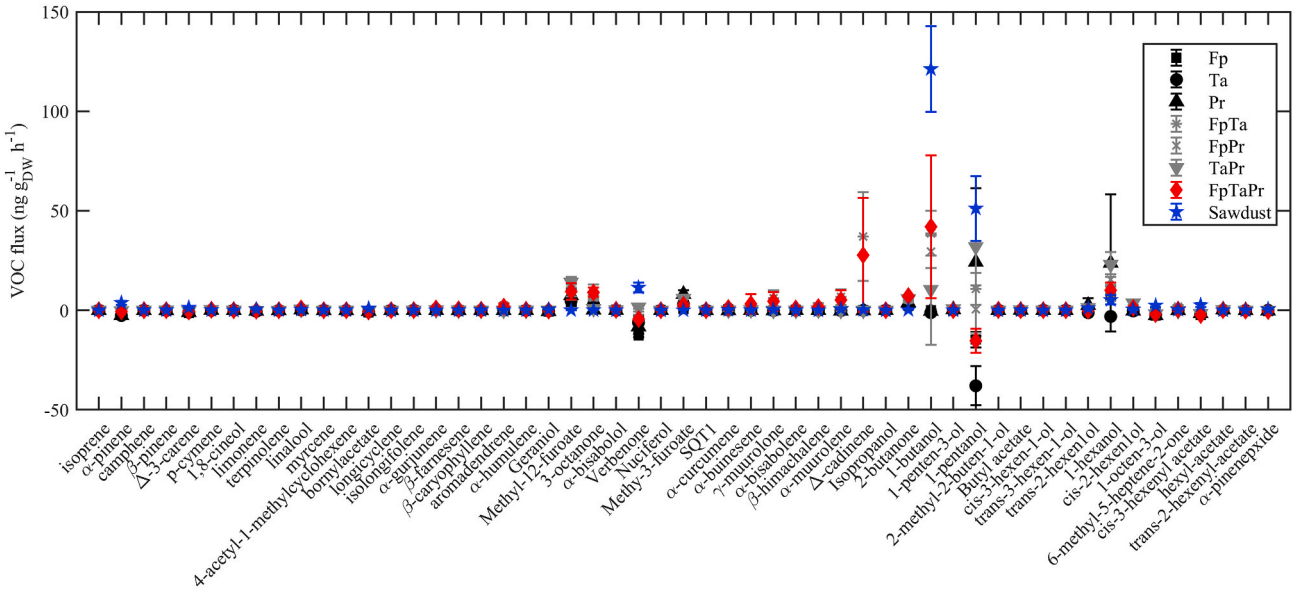

Fig. 5. Release of individual volatile organic compounds (VOCs) by the fungal cultures growing individually (Fp = *Fomitopsis pinicola*, Ta = *Trichaptum abietinum*, and Pr=*Phlebia radiata*) and in combinations on pine sawdust. Flux rates are mean values with standard deviations of four biological replicates and the flux rate of each replicate is the mean value of the three measurement times (weeks 4, 8, and 12).

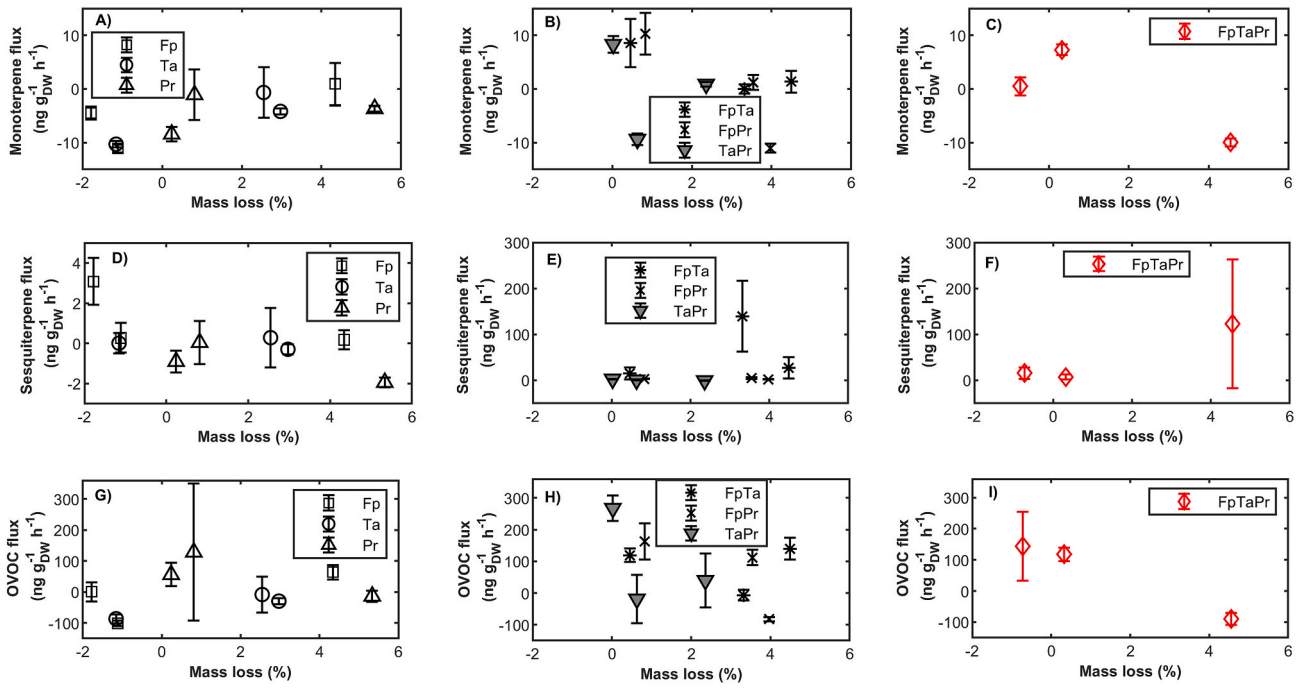

Fig. 6. The relationship between wood mass loss and release on monoterpenoids (A–C), sesquiterpenes (D–F), and oxygenated volatile organic compounds (oVOCs) (G–I) from the fungal cultures on pine sawdust. Fungal species were cultivated individually (Fp = *Fomitopsis pinicola,* Ta = *Trichaptum abietinum*, and Pr=*Phlebia radiata*) and in combinations. The mean values with standard deviations show variation of the four biological replicates during the cultivation weeks.

fungal cultures on spruce sawdust (Fig. 4). In fact, β-glucosidase activity was highest for white-rot fungus Pr on pine substrate. Fungal biomass along with the two enzyme (acidic peptidase and chitinase) activities reached their highest values in the two-species brown rot–white rot combinatory cultures on pine sawdust. The increasing trend of values in the course of the cultivation time is notable. The three-species combination showed a similar increase but with higher deviation between the four parallel culture flasks.

3.3. Factors that direct fungal VOC release on spruce substrate

We tested whether fungal species combination, ergosterol content, enzyme activities (β-glucosidase, chitinase, laccase, manganese peroxidase (MnP), and acidic peptidase), iron reduction, oxalic acid production, and amount of chelated oxalate anions (oxalate) in wood or wood mass loss could be used to explain VOC release from spruce wood by the wood-decaying fungi (Table 2). Iron reduction explained the total sesquiterpene release, whereas chelated oxalate anion content, iron reduction, and mass loss explained the total monoterpene release. Individual VOCs were also well explained by varying mixtures of fungal metabolic parameters.

Sesquiterpene release was highest on the spruce substrate, when chitinase activity was highest in the fungal cultures including the combination of brown-rot and white-rot species in pairs ($R^2 = 0.27–0.34$, $p < 0.001$***, Supplementary Table S1). Oxygenated VOC release was highest, however, when chitinase activity was lowest in the same fungal cultures ($R^2 = 0.32–0.94$, $p < 0.001$***). Monoterpene release was also highest when chitinase activity was highest in the paired co-culture of the brown-rot Fp and the white-rot Pr species ($R^2 = 0.56$, $p < 0.001$***). The exponential regression of chitinase activity and VOC release was mainly low when fungal species were growing individually.

Oxygenated VOC release was highest when fungal hyphal biomass (ergosterol content) was lowest on the spruce substrate (Supplementary Table S1). We observed a statistically significant relationship between the oxygenated VOC fluxes and ergosterol content in fungal cultures where brown-rot and white-rot species interacted with each other ($R^2 = 0.65–0.85$, $p < 0.001$***). Monoterpene release was highest when fungal hyphal biomass was highest in the paired cultures of brown-rot and white-rot species ($R^2 = 0.51–0.72$, $p < 0.001$***). Sesquiterpene release also increased with increasing ergosterol content in these paired fungal cultures ($R^2 = 0.15–0.28$, $p < 0.001$***). We also found a positive relationship between the total monoterpene and sesquiterpene fluxes and β-glucosidase activity in the FpPr combination fungal cultures ($R^2 = 0.41$ and $R^2 = 0.83$ $p < 0.001$***). Monoterpene and sesquiterpene release was highest when iron reduction activity was highest in the paired cultures of brown-rot and white-rot species ($R^2 = 0.32–0.70$, $p < 0.01$**).

3.4. Factors that direct fungal VOC release on pine substrate

Based on the linear mixed-effects models, β-glucosidase activity, chelated oxalate anion content (oxalate), and chitinase explained the total sesquiterpene release, whereas ergosterol and chelated oxalate anion content (oxalate) explained the total monoterpenenoid release on pine substrate (Table 3). Isoprene and the individual monoterpenoids (β-pinene and limonene), sesquiterpenes (SQT1*, α-bisabolene, α-curcumene and α-buinesene), and oVOCs (nuciferol and butyl acetate) were strongly explained by varying mixtures of the fungal metabolic parameters (Table 3). The explanation powers of the models were stronger on pine substrate compared to results obtained on spruce substrate.

Similar to spruce substrate, sesquiterpene release was also highest on pine substrate when chitinase activity was highest in fungal cultures, where brown-rot and white-rot species were growing in pairs ($R^2 = 0.35$, $p < 0.001$***) or all three species were growing together ($R^2 = 0.46$, $p < 0.001$***, Supplementary Table S2). The total monoterpene release on pine wood was highest when living fungal hyphal biomass (ergosterol content) was lowest in these fungal paired and tripled cultures ($R^2 = 0.19–0.37$, $p < 0.001$***).

3.5. The effect of substrate on VOC release

The measured and modelled fluxes of oVOCs, monoterpenoids, and sesquiterpenes were strongly affected by substrate quality, as the fluxes together were higher on the pine wood substrate compared to spruce wood substrate (Fig. 7).

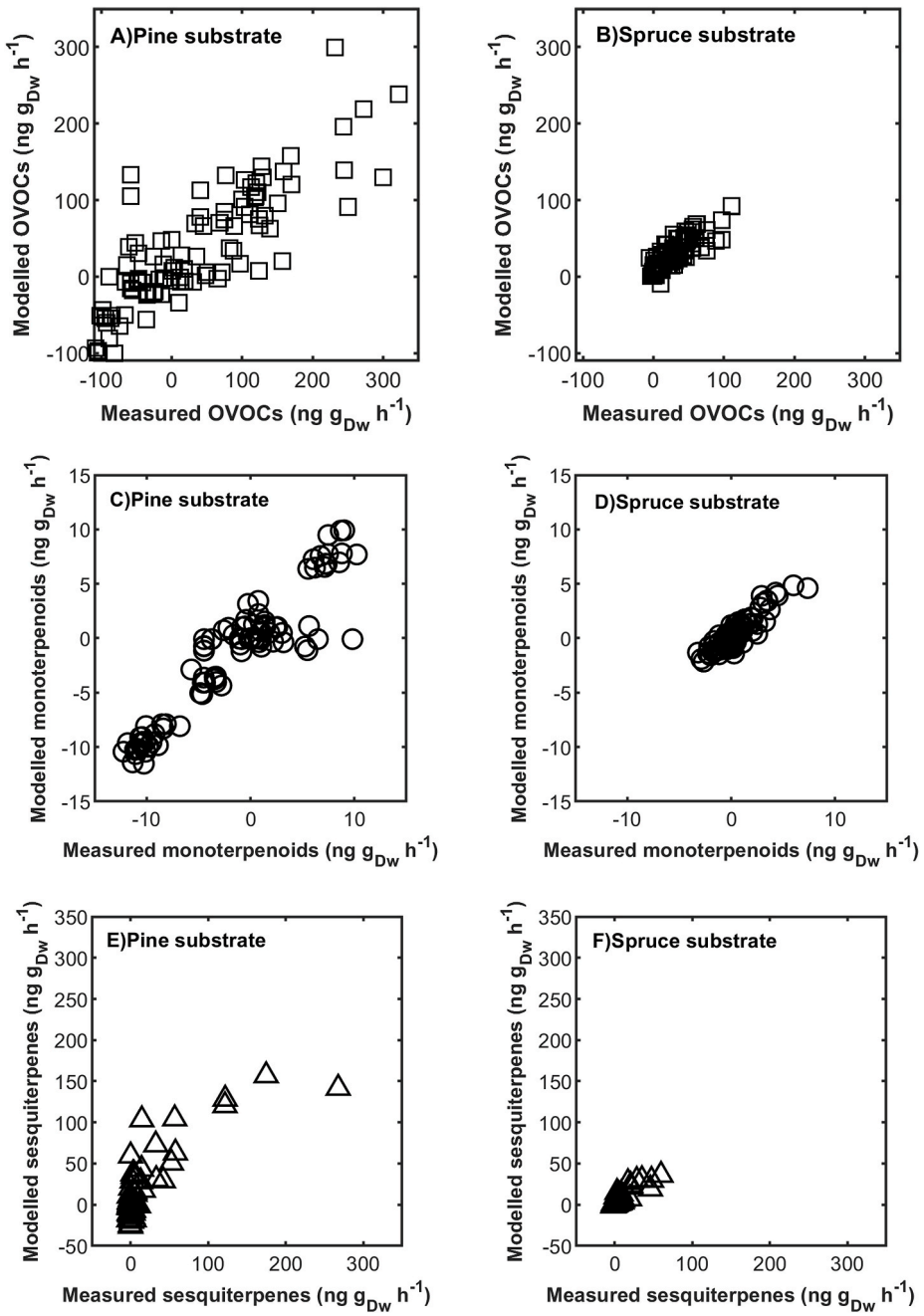

Fig. 7. The effect of substrate on modelled and measured release of oxygenated volatile organic compounds (oVOCs) (A–B), monoterpenoids (C–D), and sesquiterpenes (E–F).

4. Discussion

4.1. Fungal species interactions observed in VOC release

Our results show that VOC release from Norway spruce and Scots pine deadwood increases when wood-decaying fungi grow into the substrate compared to VOC release from uncolonized wood sawdust, indicating that fungal metabolic activity and wood decomposition were the main VOC sources and sinks. Fungal cultures released oVOCs, with low molecular weights and high volatilities, mainly at the beginning of the experiment, while less volatile monoterpenoids and sesquiterpenes with higher molecular weight were emitted during the experimental cultivation (over a 12-week period). Deadwood and woody litter contain isoprenoid storage pools, which are released into the lower atmosphere during natural decomposition in forests (Kainulainen and Holopainen 2002; Mäki et al. 2017). Wood substrate sawdust itself likely released monoterpenoids and sesquiterpenes initially. Later, following fungal hyphal growth and metabolism, the biological processes were likely the main source of VOCs from the deadwood substrates. In boreal forest soils, the highest concentrations of volatile monoterpenes and sesquiterpenes were observed in the O- and A-horizons, (Mäki et al. 2019c), where the amount of organic nitrogen and carbon, and litter (with VOC storage pools), and roots (with associated microbes) were likely at the highest levels. VOC release by saprobic microbes is crucial, as monoterpenes influence decomposition processes in the boreal soils (Adamczyk et al. 2018).

We also observed indications of fungal bioconversion or uptake (metabolized either extracellularly or intracellularly) of VOCs in this study. White-rot fungi have a remarkable ability to modify aromatic lignin-like compounds extracellularly by the aid of their versatile

oxidoreductase enzymes (Floudas et al. 2012; Lundell et al. 2014). This is a type of "detoxification" ability. VOC uptake in soils by ectomycorrhizal fungi is a crucial process in soil VOC exchange and is regulated by temperature and soil water content (Trowbridge et al. 2020). However, the observed decrease in monoterpenoid concentrations in the course of our cultivations does not necessarily indicate fungal uptake of monoterpenoids. The white-rot species have strong oxidative redox enzyme secretion capacities (laccases, manganese peroxidases, lignin peroxidases, hydrogen peroxide-producing enzymes) (Floudas et al. 2012; Lundell et al. 2014; Kuuskeri et al. 2016; Mali et al. 2017) and may thereby transform soluble phenolic monoterpenoids into oxidized products and coupled polymers. Terpenoid compounds are not utilized by wood-decaying fungi; their main sources for carbon and energy are the soluble carbohydrates of plant biomass polysaccharides (cellulose, hemicelluloses, pectins) that are produced during fungal enzymatic and biochemical decomposition processes.

An earlier study (Bäck et al. 2010) found fungi of Ascomycota and Basidiomycota growing within Scots pine roots to emit sesquiterpenes. In our current study, fungal VOC release was highest for sesquiterpenes, such as α-curcumene, α-muurolene, and Δ-cadinene, and for oVOCs, such as methyl-12-furoate, methyl-3-furoate, 3-octanone, 2-butanone, α-bisabolol, and 1-hexanol, on pine and spruce substrates. Sesquiterpenes were also reported as the dominant group of volatile compounds released during wood-inhabiting fungal interactions on beech wood (El Ariebi et al. 2016; Gramss, 2020). This observation is interesting as deciduous deadwood of beech (*Fagus sylvatica*) and coniferous deadwood like pine (*P. sylvestris*) harbour distinct fungal and prokaryotic microbial communities. The same fungal sesquiterpenes that we observed on coniferous wood (Δ-cadinene, α-muurolene, and α-gurjunene) were also observed on beech wood (Gramss, 2020), which further supports the conclusion that sesquiterpenes were mainly released by fungal metabolism of wood. Basidiomycota mycelia may produce and release sesquiterpenes during inter-species interactions (Kramer and Abraham 2012). Similar to our approach, the brown-rot species *F. pinicola* was paired with a white-rot fungus (*Kuehneromyces mutabilis*) (Gramss, 2020), and likewise, we found that sesquiterpene release showed a strong increase in the dual cultures of *F. pinicola* and a white-rot species, either *P. radiata* or *T. abietinum*.

In a past study, we observed that interactions of the brown-rot species *F. pinicola* with white-rot fungi accelerate VOC release on spruce wood compared to single-species cultivations (Mali et al. 2019). In our current study, interactions of *F. pinicola* with the white-rot species *T. abietinum* and *P. radiata* accelerated sesquiterpene release during fungal growth and decomposition on both substrates, i.e. Norway spruce and Scots pine wood sawdust. Sesquiterpene release by species combinations FpTa and FpTaPr was exceptionally high during the second measurement month on pine substrate. Wood decomposition activity was higher in fungal co-cultures on spruce substrate compared to single-species cultures (Mali et al. 2019).

Our previous studies with these fungal species (*F. pinicola*, *P. radiata*, *T. abietinum*) and the same isolates on agar medium plates and in liquid media demonstrate their co-existence and success upon hyphal interactions (Mali et al. 2017). However, it is possible that the fungal hyphal antagonistic contacts and competition during the second cultivation month may have led to species replacement or partial replacement in the combinatory cultures. Antagonistic interactions between wood-decaying fungi and competition for territory may lead to a deadlock or to full, partial, or mutual replacement, and involve the release of volatile compounds (Boddy 2016). In our current study, sesquiterpene and monoterpene release by *F. pinicola* in individual cultures and in pairs with white-rot species on spruce substrate was low during the first month but subsequently increased. Surprisingly, El Ariebi et al. (2016) found that VOC-driven effects on mycelia extension rates were stronger in intraspecific interactions compared to interspecific interactions. Quantitative and qualitative release of VOCs was highest in interspecific (involving two species) interactions (El Ariebi et al. 2016). However, microbial VOCs occurring in the highest abundance may not demonstrate the highest impact for other microbes (Romano et al. 2015). Moreover, higher microbial diversity in shared habitats may not necessarily lead to an increase in VOC emissions.

4.2. Wood substrate influences VOC release by fungi

Our results show that VOC synthesis and release by wood-decaying fungi depend on their growth substrate (either spruce or pine wood). Wood substrate influenced the VOC release by the fungi, as the measured fluxes of oVOCs and sesquiterpenes were clearly higher on pine sawdust compared to spruce sawdust. Substrate also affects forest floor VOC fluxes because pine (*P. sylvestris*) deadwood dominated floor was found to be a stronger VOC source than spruce (*P. abies*) deadwood dominated forest floor in both boreal and hemiboreal climate locations (Mäki et al. 2019b). Monoterpene release from decomposing pine litter was five to ten times faster than from decomposing spruce litter at an early stage of decomposition (Isidorov et al., 2010). In addition, filamentous fungi may generate various VOCs as metabolic by-products under less favourable mycelial growth conditions (Busko et al., 2016). Taken together, the higher amount of VOCs generated in pine wood cultures may result from VOCs released from wood and generated by the fungi.

In our study, monoterpene release was highest during the first month of growth on pine substrate, whereas the highest emission of monoterpenes on spruce wood occurred during the second and third measurement months, which implies that substrate quality affects both hyphal growth and VOC release and synthesis by wood-decaying fungi. On the other hand, we observed a decreasing pattern of oVOC emissions by the brown-rot fungus *F. pinicola* along with white-rot fungus *T. abietinum* on pine substrate. These results indicate either further modification of the oVOCs or potential uptake of the original compounds by the fungi, depending on the wood substrate (pine vs. spruce). In line with our results, VOC composition has been found to depend on both the fungal species and deadwood substrate (pine vs. spruce) (Isidorov et al. 2016). VOC release may additionally vary due to diversity of microbial taxa, the life stage of the tested microbes, and substrate type (Misztal et al. 2018).

4.3. Indicators of fungal metabolism correlate with VOC release

According to our results, interactions between brown-rot and white-rot fungi may lead to an increase in extracellular chitinase activity, which in turn correlated with an accelerated release of isoprenoids. The total sesquiterpene fluxes were highest in the combinatory cultures of FpTa and FpTaPr on spruce wood substrate, and chitinase activity peaked at the end of the cultivation period (Mali et al. 2019). Chitinases are secreted enzymes capable of degrading fungal cell wall chitin polymers into oligosaccharides and amino sugars (Ekenler and Tabatabai 2002; Adamczyk et al. 2015). We found a positive relationship between sesquiterpene and monoterpene release and chitinase activity when *F. pinicola* and white-rot species *P. radiata* were growing together on spruce wood. A similar trend was observed on pine wood substrate, as chitinase activity increased in parallel to sesquiterpene release in the co-cultures of a brown-rot (Fp) and a white-rot fungus (Pr). These results indicate that enzymatic attack of opposing fungi against each other's mycelium may increase sesquiterpene release by wood-decaying fungi. Our results are supported by another study indicating that the production of chitinase activity increased at the hyphal interaction zone (Hiscox and Boddy 2017).

Our results indicate that interactions of the brown-rot fungus *F. pinicola* and the white-rot fungi may either enhance or suppress fungal extracellular enzyme activities, as observed in our previous study (Mali et al. 2017). Living fungal biomass (ergosterol content), iron reduction activity, and β-glucosidase activity increased together with the total monoterpene and sesquiterpene fluxes in fungal dual cultures of *F.*

pinicola and *P. radiata* on spruce wood. β-glucosidases are enzymes with versatile functions in fungi, and they are needed in the depolymerization of cellulose- and hemicellulose-derived oligosaccharides to glucose (Rytioja et al. 2014). We also observed a negative relationship between the total oVOC fluxes and β-glucosidase activity and between the total oVOC fluxes and iron reduction activity in fungal cultures on spruce substrate, where the brown-rot and white-rot species were combined.

These results indicate that space and resource competition, and oVOCs may have an antagonistic effect on the enzyme activities or production by fungal mycelia. Negative impacts of VOCs on microbial activity have previously been observed. For example, isoprenoids inhibited microbial-secreted enzyme activities (chitinase and β-glucosidase) in laboratory studies (Adamczyk et al. 2015). Another explanation is that fungi released oVOCs at the beginning of cultivations, when β-glucosidase activity was still low. This is supported by findings showing strong oxidative bursts generated by wood-decaying brown-rot fungi (by Fenton reactions) and white-rot fungi (by secretion of oxidoreductase enzymes attacking lignin or generating H_2O_2) at an early phase of hyphal growth and wood decomposition (Kuuskeri et al. 2016; Zhang et al. 2016). Chemical and enzymatic oxidation reactions may also convert the wood-released VOCs.

4.4. Modelling fungal VOC release with fungal metabolism indicators

It is challenging to separate the effect of fungal metabolism indicators on fungal VOC release from the effect of various VOCs on fungal activities and metabolism. We used linear mixed-effects models to determine these relationships, and found that fungal metabolism indicators partly explained the fungal VOC release from wood. Iron reduction activity explained 71% of the release of sesquiterpenes, whereas the amounts of chelated oxalate anions, iron reduction, and wood mass loss explained 78% of the release of monoterpenenoids on spruce wood substrate. Iron reduction capacity indicates Fenton chemistry and the brown-rot decay of wood, and is generated in cultures of *F. pinicola* (Shah et al. 2018). Together with iron reduction capacity, a strong secretion of oxalic acid is typical for *F. pinicola* (Mali et al. 2017; Shah et al. 2018), and most of the secreted oxalic acid is found as oxalate anions attached to wood in the later stages of cultivation on solid wood (Mali et al. 2019; Villavicencio et al. 2020).

Our results are supported by another study with *F. pinicola* subjecting the brown-rot fungus in dual cultures with a white-rot species (*K. mutabilis*) demonstrating oxalic acid production to support the acidification of the substrate (Gramss et al. 2020). As *F. pinicola* is able to survive at very low pH (below pH 2) (Mali et al. 2017; Shah et al. 2018), it is also able to control monoterpene and sesquiterpene concentrations in the headspace air of the wood substrate cultures (Gramss, 2020). Fungal oxalate is a strong chelator, which also enables dissolving and release of bound iron and other cations from decomposing wood (Arentes et al. 2009). Wood substrate appears to influence the relationship of fungal VOC release and fungal metabolism indicators, especially the release of sesquiterpenes and monoterpenoids. To conclude, fungal VOC release was more strongly driven by fungal metabolism indicators on pine wood substrate compared to spruce wood.

Individual VOCs were well explained by varying the mixture of fungal metabolic parameters, indicating that production processes of different VOCs are divergent. Enzyme activities of chitinase, β-glucosidase, laccase, and manganese peroxidase were found to explain the VOC release of individual compounds. In another study, laccase and manganese peroxidase activities and production decreased during fungal interspecies mycelia deadlock compared to partial replacement during fungal interactions (O'Leary et al. 2019). Activities of chitinase and β-glucosidase also decreased slightly during terpene exposure (Adamczyk et al. 2015). These notions imply that VOCs may interfere and inhibit crucial activities of wood-decomposing enzymes. Competition between fungal species apparently lead to metabolic changes, along with variation in wood decomposition rates, which differ depending on the interaction partners (Hiscox et al. 2015).

In our study, the limited temporal coverage of the measurements may have hindered the detection of immediate interaction responses between the fungal species. On the other hand, our experiment, lasting three months under controlled conditions, provides indications of longer-term biological and chemical changes occurring in the decaying wood. As abiotic factors, such as temperature, may affect the outcome of fungal interactions (Hiscox and Boddy 2017), the extension of species-combinatory experiments to forest sites and natural habitats of the fungi is crucial. In situ measurements, where seasonal effects are detected in changes in relative humidity, temperature, and soil moisture conditions, vary in time and space and will provide valuable forest ecological information.

5. Conclusions

This study shows that deadwood substrate influences VOC released by wood-decaying fungi, with signature differences caused by the decomposition trait, either brown-rot or white-rot type, and species-species interactions. Our results imply that fungal metabolism indicators, such as fungal biomass, wood decomposition activity, iron reduction, extracellular enzyme activities, oxalate anion content, and oxalic acid production, may partly explain the VOC release of wood-decaying Basidiomycota. A warming climate could stimulate these interactions and the growth of certain, dominating fungi, e.g. *F. pinicola*, in their natural habitats. Furthermore, the release of more CO_2 into the atmosphere as a consequence of accelerated heterotrophic respiration, or an increase in sesquiterpene emissions from decaying wood will both influence air chemistry under the canopy and the overall carbon cycle in forest ecosystems.

Author contribution

Constructing and designing the study: all authors (MM, TM, HH, JH, TL, and JB). Fungal cultivations: TM. Collection of VOC samples: MM, TM. VOC analyses: HH, MM. Enzyme activity, fungal metabolite, and wood mass loss analyses: TM. Statistical analyses: MM. Preparing the manuscript: MM. Supervision: TL, JH, JB. All authors participated in the experimental design, interpretation of the results, and in writing the manuscript.

Disclaimer

We the authors confirm no competing financial interests. We also affirm that this manuscript has not been submitted elsewhere.

Data availability statement

We added all the data published in this article to the supplementary files.

Acknowledgements

We thank and acknowledge the funding from Jenny and Antti Wihuri Foundation (to MM), the study position of the Doctoral Programme for Microbiology and Biotechnology, University of Helsinki (to TM), the Academy of Finland Centre of Excellence programme (project no **272041**), the Academy of Finland project Fungcolife (project no **285676**), and the Jane and Aatos Erkko foundation grant (no **170101** to TL).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2021.101106.

References

Aaltonen, H., Pumpanen, J., Pihlatie, M., Hakola, H., Hellén, H., Kulmala, L., Vesala, T., Bäck, J., 2011. Boreal pine forest floor biogenic volatile organic compound fluxes peak in early summer and autumn. Agric. For. Meteorol. 151, 682–691.

Adamczyk, S., Adamczyk, B., Kitunen, K., Smolander, A., 2015. Monoterpenes and higher terpenes may inhibit enzyme activities in boreal forest soil. Soil Biol. Biochem. 87, 59e66.

Adamczyk, B., Adamczyk, S., Smolander, A., Kitunen, K., Simon, J., 2018. Plant secondary metabolites—missing pieces in the soil organic matter puzzle of boreal forests. Soil Systems 2, 2.

Arentes, V., Qian, Y., Milagres, A.M.F., Jellison, J., Goodell, B., 2009. Effect of pH and oxalic acid on the reduction of Fe^{3+} by a biomimetic chelator and on Fe^{3+} desorption/adsorption onto wood: implications for brown-rot decay. Int. Biodeterior. Biodegrad. 63, 478–483.

Bäck, J., Aaltonen, H., Hellén, H., Kajos, M.K., Patokoski, J., Taipale, R., Pumpanen, J., Heinonsalo, J., 2010. Variable fluxes of microbial volatile organic compounds (MVOCs) from root associated fungi isolated from Scots pine. Atmos. Environ. 44, 3651–3659.

Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., Tonon, G., Brusetti, L., 2018. The role of microbial community in the decomposition of leaf litter and deadwood. Appl. Soil Ecol. 126, 75–84.

Boddy, L., 2000. Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbial Ecology 31, 185–194.

Boddy, L., 2016. Interactions between fungi and other microbes. In: Watkinson, S.C., Boddy, L., Money, N.P. (Eds.), The Fungi. Elsevier, London, p. 340.

Busko, M., Stuper, K., Jelen, H., Góral, T., Chmielewski, J., Tyrakowska, B., Perkowski, J., 2016. Comparison of volatiles profile and contents of trichothecenes group B, ergosterol, and ATP of bread wheat, Durum wheat, and Triticale grain naturally contaminated by mycobiota. Front. Plant Sci. 7, 1243.

Classen, A.T., Sundqvist, M.K., Henning, J.A., Newman, G.S., Moore, J.A.M., Cregger, M. A., Moorhead, L.C., Patterson, C.M., 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: what lies ahead? Ecosphere 6, 130.

Ditengou, F.A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., van Doorn, M.M., Legue, V., Palme, K., Schnitzler, J.P., Polle, A., 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nat. Commun. 6, 6279.

Eastwood, D.C., Floudas, D., Binder, M., Majcherczyk, A., Schneider, P., Aerts, A., Asiegbu, F.O., Baker, S.E., Barry, K., Bendiksby, M., Blumentritt, M., Coutinho, P.M., Cullen, D., de Vries, R.P., Gathman, A., Goodell, B., Henrissat, B., Ihrmark, K., Kauserud, H., Kohler, A., LaButti, K., Lapidus, A., Lavin, J.L., Lee, Y.H., Lindquist, E., Lilly, W., Lucas, S., Morin, E., Murat, C., Oguiza, J.A., Park, J., Pisabarro, A.G., Riley, R., Rosling, A., Salamov, A., Schmidt, O., Schmutz, J., Skrede, I., Stenlid, J., Wiebenga, A., Xie, X., Kües, U., Hibbett, D.S., Hoffmeister, D., Högberg, N., Martin, F., Grigoriev, I.V., Watkinson, S.C., 2011. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science 333, 762–765.

Ekenler, M., Tabatabai, M.A., 2002. Effects of trace elements on betaglucosaminidase activity in soils. Soil Biol. Biochem. 32, 1829e1832.

El Ariebi, N., Hiscox, J., Scriven, S.A., Müller, C.T., Boddy, L., 2016. Production and effects of volatile organic compounds during interspecific interactions. Fungal Ecology 20, 144–154.

Evans, J.A., Eyre, C.A., Rogers, H.J., Boddy, L., Müller, C.T., 2008. Changes in volatile production during interspecific interactions between four wood rotting fungi growing in artificial media. Fungal Ecology 1, 57–68.

Ewen, R.J., Jones, P.R.H., Ratcliffe, N.M., Spencer-Phillips, P.T.N., 2004. Identification by gas chromatography-mass spectrometry of the volatile organic compounds emitted from the wood-rotting fungi *Serpula lacrymans* and *Coniophora puteana*, and from *Pinus sylvestris* timber. Mycol. Res. 108, 806–814.

Floudas, D., Binder, M., Riley, R., Berry, K., Blanchette, R.A., Henrissat, B., Martínez, A. T., Otillar, R., Spatafora, J.W., Yadav, J.S., Aerts, A., Benoit, I., Boyd, A., Carlson, A., Copeland, A., Coutinho, P.M., de Vries, R.P., Ferreira, P., Findley, K., Foster, B., Gaskell, J., Glotzer, D., Górecki, P., Heitman, J., Hesse, C., Hori, C., Igarashi, K., Jurgens, J.A., Kallen, N., Kersten, P., Kohler, A., Kües, U., Kumar, T.K., Kuo, A., LaButti, K., Larrondo, L.F., Lindquist, E., Ling, A., Lombard, V., Lucas, S., Lundell, T., Martin, R., McLaughlin, D.J., Morgenstern, I., Morin, E., Murat, C., Nagy, L.G., Nolan, M., Ohm, R.A., Patyshakuliyeva, A., Rokas, A., Ruiz-Dueñas, F.J., Sabat, G., Salamov, A., Samejima, M., Schmutz, J., Slot, J.C., St John, F., Stenlid, J., Sun, H., Sun, S., Syed, K., Tsang, A., Wiebenga, A., Young, D., Pisabarro, A., Eastwood, D.C., Martin, F., Cullen, D., Grigoriev, I.V., Hibbett, D.S., 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336, 1715–1719.

Gramss, G., 2020. Aspects determining the dominance of *Fomitopsis pinicola* in the colonization of deadwood and the role of the pathogenicity factor oxalate. Forests 11, 290.

Hellén, H., Praplan, A.P., Tykkä, T., Ylivinkka, I., Vakkari, V., Bäck, J., Petäjä, T., Kulmala, M., Hakola, H., 2018. Long-term measurements of volatile organic compounds highlight the importance of sesquiterpenes for the atmospheric chemistry of a boreal forest. Atmos. Chem. Phys. 18, 13839–13863.

Hiscox, J., Boddy, L., 2017. Armed and dangerous: chemical warfare in wood decay communities. Fungal Biology Reviews 31, 169e184.

Hiscox, J., Savoury, M., Vaughan, I., Müller, C.T., Boddy, L., 2015. Antagonistic fungal interactions influence carbon dioxide evolution from decomposing wood. Fungal Ecology 14, 24–32.

Hiscox, J., O'Leary, J., Boddy, L., 2018. Fungus wars: basidiomycete battles in wood decay. Stud. Mycol. 89, 117–124.

Hoppe, B., Purahong, W., Wubet, T., Kahl, T., Bauhus, J., Arnstadt, T., Hofrichter, M., Buscot, F., Krüger, D., 2016. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in Central European forests. Fungal Divers. 77, 367–379.

Isidorov, V.A., Smolewska, M., Purzynska-Pugacewicz, A., Tyszkiewicz, Z., 2010. Chemical composition of volatile and extractive compounds of pine and spruce leaf litter in the initial stages of decomposition. Biogeosciences 7, 2785–2794.

Isidorov, V., Tyszkiewicz, Z., Pirożnikov, E., 2016. Fungal succession in relation to volatile organic compounds emissions from Scots pine and Norway spruce leaf litter-decomposing fungi. Atmos. Environ. 131, 301–306.

Kainulainen, P., Holopainen, J.K., 2002. Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. Soil Biol. Biochem. 34, 37–42.

Kramer, R., Abraham, W.R., 2012. Volatile sesquiterpenes from fungi: what are they good for? Phytochemical Review 11, 15–37.

Kuuskeri, J., Mäkelä, M.R., Isotalo, J., Oksanen, I., Lundell, T., 2015. Lignocellulose-converting enzyme activity profiles correlate with molecular systematics and phylogeny grouping in the incoherent genus *Phlebia* (Polyporales, Basidiomycota). BMC Microbiol. 15, 1–18.

Kuuskeri, J., Häkkinen, M., Laine, P., Smolander, O.P., Tamene, F., Miettinen, S., Nousianen, P., Kemell, M., Auvinen, P., Lundell, T., 2016. Time-scale dynamics of proteome and transcriptome of the white-rot fungus *Phlebia radiata*: growth on spruce wood and decay effect on lignocellulose. Biotechnol. Biofuels 9, 192.

Lundell, T.K., Mäkelä, M.R., de Vries, R.P., Hildén, K.S., 2014. Genomics, lifestyles and future prospects of wood-decay and litter-decomposing Basidiomycota. Adv. Bot. Res.: Fungi 70, 329–370.

Mäki, M., Heinonsalo, J., Hellén, H., Bäck, J., 2017. Contribution of understorey vegetation and soil processes to boreal forest isoprenoid exchange. Biogeosciences 14, 1055–1073.

Mäki, M., Aalto, J., Hellén, H., Pihlatie, M., Bäck, J., 2019a. Interannual and seasonal dynamics of volatile organic compound fluxes from the boreal forest floor. Front. Plant Sci. 10, 191.

Mäki, M., Krasnov, D.J., Hellén, H., Noe, S.M., Bäck, J., 2019b. Stand type affects forest floor fluxes of volatile organic compounds in hemiboreal and boreal climates. Plant Soil 441, 363–381.

Mäki, M., Aaltonen, H., Heinonsalo, J., Hellén, H., Pumpanen, J., Bäck, J., 2019c. Boreal forest soil is a significant and diverse source of volatile organic compounds. Plant Soil 441, 89–110.

Mali, T., Kuuskeri, J., Shah, F., Lundell, T., 2017. Interactions affect hyphal growth and enzyme profiles in combinations of coniferous wood-decaying fungi of Agaricomycetes. PloS One 12, e0185171.

Mali, T., Mäki, M., Hellén, H., Heinonsalo, J., Bäck, J., Lundell, T., 2019. Decomposition of spruce wood and release of volatile organic compounds depend on decay type, fungal interactions and enzyme production patterns. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 95 fiz135.

Mattila, H., Kuuskeri, J., Lundell, T., 2017. Single-step, single-organism bioethanol production and bioconversion of lignocellulose waste materials by phlebioid fungal species. Bioresour. Technol. 225, 254–261.

Misztal, P.K., Lymperopoulou, D.S., Adams, R.I., Scott, R.A., Lindow, S.E., Bruns, T., Taylor, J.W., Uehling, J., Bonito, G., Vilgalys, R., Goldstein, A.H., 2018. Emission factors of microbial volatile organic compounds from environmental bacteria and fungi. Environ. Sci. Technol. 52, 8272–8282.

Müller, A., Faubert, P., Hagen, M., zu Castell, W., Polle, A., Schnitzler, J.P., Rosenkranz, M., 2013. Volatile profiles of fungi - chemotyping of species and ecological functions. Fungal Genet. Biol. 54, 25–33.

O'Leary, J., Hiscox, J., Eastwood, D.C., Savoury, M., Langley, A., McDowell, S.W., Roger, H.J., Boddy, L., Müller, C.T., 2019. The whiff of decay: linking volatile production and extracellular enzymes to outcomes of fungal interactions at different temperatures. Fungal Ecology 39, 336–348.

Rajala, T., Peltoniemi, M., Pennanen, T., Makipää, R., 2012. Fungal community dynamics in relation to substrate quality of decaying Norway spruce *(Picea abies* L. Karst.) logs in boreal forests. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 81, 494–505.

Romano, A., Capozzi, V., Spano, G., Biasiolo, F., 2015. Proton transfer reaction–mass spectrometry: online and rapid determination of volatile organic compounds of microbial origin. Appl. Microbiol. Biotechnol. 99, 3787–3795.

Rytioja, J., Hildén, K., Hatakka, A., Mäkelä, M.R., 2014. Transcriptional analysis of selected cellulose-acting enzymes encoding genes of the white-rot fungus *Dichomitus squalens* on spruce wood and microcrystalline cellulose. Fungal Genet. Biol. 72, 91–98.

Schenkel, D., Maciá-Vicente, J.G., Bissell, A., Splivallo, R., 2018. Fungi indirectly affect plant root architecture by modulating soil volatile organic compounds. Front. Microbiol. 9, 1847.

Schulz-Bohm, K., Martín-Sánchez, L., Garbeva, P., 2017. Microbial volatiles: small molecules with an important role in intra- and inter-kingdom interactions. Front. Microbiol. 8, 2484.

Shah, F., Mali, T., Lundell, T.K., 2018. Profiles of enzyme activities, production of oxalic acid, and Fe^{3+} -reducing metabolites secreted by the Polyporales brown-rot species *Fomitopsis pinicola*. Appl. Environ. Microbiol. 84 e02662-17.

Smolander, A., Kanerva, S., Adamczyk, B., Kitunen, V., 2012. Nitrogen transformations in boreal forest soils – does composition of plant secondary compounds give any explanations? Plant Soil 350, 1–26.

Trowbridge, A.M., Stoy, P.C., Phillips, R.P., 2020. Soil biogenic volatile organic compound flux in a mixed hardwood forest: net uptake at warmer temperatures and

the importance of mycorrhizal associations. J. Geophys. Res.: Biogeosciences 124, 1–14.
Villavicencio, E.V., Mali, T., Mattila, H.K., Lundell, T., 2020. Enzyme activity profiles produced on wood and straw by four fungi of different decay strategies. Microorganisms 8, 73.
Zhang, J., Presley, G.N., Hammel, K.E., Ryu, J.S., Menke, J.R., Figueroa, M., Hu, D., Orr, G., Schilling, J.S., 2016. Localizing gene regulation reveals a staggered wood decay mechanism for the brown rot fungus *Postia placenta*. Proc. Natl. Acad. Sci. Unit. States Am. 113, 10968–10973.