



## Tree species replacement from birch to spruce affects eukaryome in boreal forest soil

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### ABSTRACT

Large-scale replacements of native birch with spruce have been carried out in Western Norway for economic reasons. This tree species shift potentially affects biotic components such as the eukaryome, consisting of microscopic animals (Metazoa), protists and fungi, which are key players in the functioning of forest ecosystem. The impact on the belowground eukaryome and its interactions with vegetation and soil properties is not well assessed. We examined the impact of replacing native birch with Norway spruce plantations on the eukaryome of the boreal forest floor in Western Norway using 18S rDNA metabarcoding. The tree species shift from birch to spruce had significant impacts on the eukaryome at both taxonomic (Metazoa) and functional categories (phagotrophs, phototrophs, parasites and osmotrophs). The distinct differences in eukaryome communities were related to changes in understorey vegetation biomass and soil chemistry following the tree species shift. This had a negative effect on eukaryome richness, particularly affecting phagotrophs and parasites, while the opposite was observed for osmotroph richness. Our results indicated that the spruce plantations altered the eukaryome communities and their food-web patterns compared to what was found in the native birch forest soil. This information should be taken into consideration in forest management planning.

Tree species shift from birch (*Betula pubescens* Ehrh.) to spruce (*Picea abies* (L.) has been carried out in Western Norway for economic purposes [1]. The tree species shift has been found to alter understorey vegetation, soil chemistry, carbon (C) stock and decomposition rates of the forest floor [2]. The shift may in turn affect soil biota (prokaryotes, microfauna, protists and algae), which are functionally diverse and vital for ecosystem functioning [3]. Different tree species may have specific relationships to fungi [4,5], protists [5], nematodes [6], arthropods and earthworms [7]. In addition, interactions between biotic (ciliates, testate amoebae, oomycetes, earthworms, nematodes, and arthropods) [5–7] and abiotic factors (e.g. moisture, pH, C, N and nutrient concentrations) [3,8] may influence the overall eukaryome diversity and communities. Despite the major importance of the eukaryome for forest ecosystem functioning, the impact of tree species changes on community patterns at taxonomic (Metazoa) and functional (phagotrophs,

phototrophs, parasites and osmotrophs) categories are largely unknown.

We selected four locations in Western Norway with adjacent, paired stands of native, naturally occurring birch and spruce planted ~45–60 years ago [2]. At each location, three replicate paired plots (144 m<sup>2</sup>) were established, and 20 forest floor cores (Litter, Fibric and Humic horizons, LFH) were sampled and bulked into one composite sample from each plot. For detailed information on locations, sampling design, and data on understorey vegetation and soil characteristics, refer [2] (Material S1); for DNA analysis methodology refer [9].

The term “eukaryome” refers to fungi, protists and microscopic Metazoa (soil animals). Operational Taxonomic Units (OTUs) were classified into taxonomic (Metazoa) and four functional categories (phagotrophs, phototrophs, parasites and osmotrophs) (Table S1) based on previous recommendation [10–12]. The Metazoa were kept as a separate taxonomic group due difficulties to assign them to the

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functional categories. We used Principal Component Analysis (PCA) to group understorey vegetation biomass (biomass PC1; dwarf shrubs and small trees, graminoids, herbs and pteridophytes) and soil chemistry (soil PC2; C/N ratio and concentrations of C, N, K and Na) (Fig. S1) [2, 4]. Keeping location as a random variable, Generalized Linear Mixed-effects Models (GLMM) were used to assess relationships between richness and various predictors (tree species, biomass PC1 and soil PC2) using the *lme4* package. Global Nonmetric Multi-Dimensional Scaling ordination and permutational analysis of variance were used to assess the relationship of overall eukaryome and different taxonomic and functional categories with the above predictors using the *vegan* package.

The overall eukaryome richness was negatively affected by the tree species shift (spruce  $534 \pm 18$ ; birch  $568 \pm 20$ ; p-value: 0.04). The negative response of the functional groups phagotrophs and parasites richness was related to soil chemistry and to both understorey vegetation biomass and soil chemistry, respectively (Table S2). Additionally, the quality of needle and leaf litter and the forest floor in spruce and birch stands was expected to play a role, as reflected in the lower litter decomposition rates (quantity) in spruce [2]. Previous studies have reported similar effects on the richness of protists [13] and arthropods [14]. In contrast, osmotrophs, showed a higher richness in spruce compared to birch stands, which was specifically related to biomass PC1.

The tree species shift mediated changes in soil chemistry and understorey vegetation species groups and biomass impacted the structure of eukaryome at taxonomic (Metazoa) and specific functional categories (phagotrophs, phototrophs and osmotrophs), whereas parasites were affected only by understorey vegetation (Table 1). Phagotrophs and parasites were more abundant in the birch stands, whereas patterns contrasted for osmotrophs and metazoans (Fig. S2). Amongst the soil chemistry parameters, especially the higher C/N ratio in spruce stands affected the community composition on all category levels except parasites (Table S3).

The metazoan community structure (Fig. 1a) was related to soil pH, %N, exchangeable Mn, Na and Co, and biomass of Cryptogams (mainly bryophytes), dominating understorey vegetation in the spruce stands (Table S3). The metazoan composition shifted from Nematoda- and copepods (Arthropoda)-domination in birch stands, to Annelida-rich and oribatid mite (Arthropoda)-domination in the slightly more acidic forest floor of spruce (Fig. 1b and c). The abundance of nematodes such as *Paratylenchus*, *Criconea* (plant feeders) and *Alaimus* (bacterivore) may be linked to the understorey vegetation species composition and higher biomass [2] in birch stands, as well as differences in tree litter

**Table 1**  
PERMANOVA analyses showing the effects of tree species, understorey vegetation biomass and soil chemistry on eukaryome community structure. Effect of tree species (birch vs spruce), understorey vegetation biomass index (biomass PC1) and soil chemistry variability index (soil PC2), on overall eukaryome, taxonomic (Metazoa) and functional categories (phagotrophs, phototrophs, parasites and osmotrophs) communities were computed using 9999 permutations. Biomass PC1 is the score of first axis of PCA conducted for the understorey biomass (Fig. S1a). Soil PC2 is the score of the second axes of the principal component analysis (PCA) carried out for soil chemical properties (Fig. S1b). Numbers in bold represent significant effects ( $p < 0.05$ ).  $R^2$  indicates correlation co-efficient.

Community structure	Tree species		Biomass PC1		Soil PC2	
	$R^2$	p value	$R^2$	p value	$R^2$	p value
Overall	0.11	<0.001	0.12	<0.001	0.09	<0.001
<b>Taxonomic group</b>	0.11	<0.001	0.12	0.009	0.10	<0.001
Metazoa						
<b>Functional categories</b>	0.10	<0.001	0.11	<0.001	0.09	0.001
Phagotrophs						
Phototrophs	0.18	<0.001	0.19	<0.001	0.14	<0.001
Parasites	0.14	<0.001	0.14	<0.001	0.08	0.055
Osmotrophs	0.12	<0.001	0.12	<0.001	0.09	0.007

quality and chemistry which in turn affect the soil and vice versa. Members of Annelida (*Marionina*) and litter-dwelling oribatid mites (*Atropacarus*, *Rhagidia* and *Allonothrus*) may occupy multiple trophic levels (i.e. decomposers, fungal feeders, scavengers and predators), thereby influencing several eukaryomes (e.g nematode and fungi) [15, 16]. Decomposition rates in spruce [2] may be altered by e.g. production of inert microarthropod excrements within the needle litter [17].

The phagotrophic communities were structured by understorey vegetation biomass, pH, %C, C/N ratio, Ba, Co, Pb, S and Si (Fig. 1d; Table S3), which agrees with previous finding [18]. Among phagotrophs, Variosea (Conosa; Amoebozoa), Stramenopiles (MAST) and Lobosa (particularly Tubulinea and Lobosa-G1) were abundant in birch, whereas Ciliophora was more common in spruce (Fig. 1e). The higher abundance of *Platyophrya* (feeding on bacteria and diatoms) [19] and *Vampyrella* (feeding on algae, fungi and nematodes) [20] in birch stand and their diverse feeding preferences indicate possible interactions at upper (tertiary consumers) and lower trophic levels (primary consumers). In spruce, Ciliophora, represented by *Pseudourostyla* (Urostyleid group) was abundant (Fig. 1f) which feeds on bacteria, ciliates and other protists.

The abundance of phagotrophs was tightly linked with phototrophs, potentially via algae as a food source [21]. Phototrophic community structure was impacted by understorey biomass and soil chemistry variables such as N, C/N and Na (Fig. 1g; Table S3). Among phototrophs, Ochrophyta were more common in birch stand whereas Chlorophyta (*Mychonastes*, *Coccomyxa*, *Elliptochloris* and *Dictyochloropsis*) dominated in spruce stands (Fig. 1h and i).

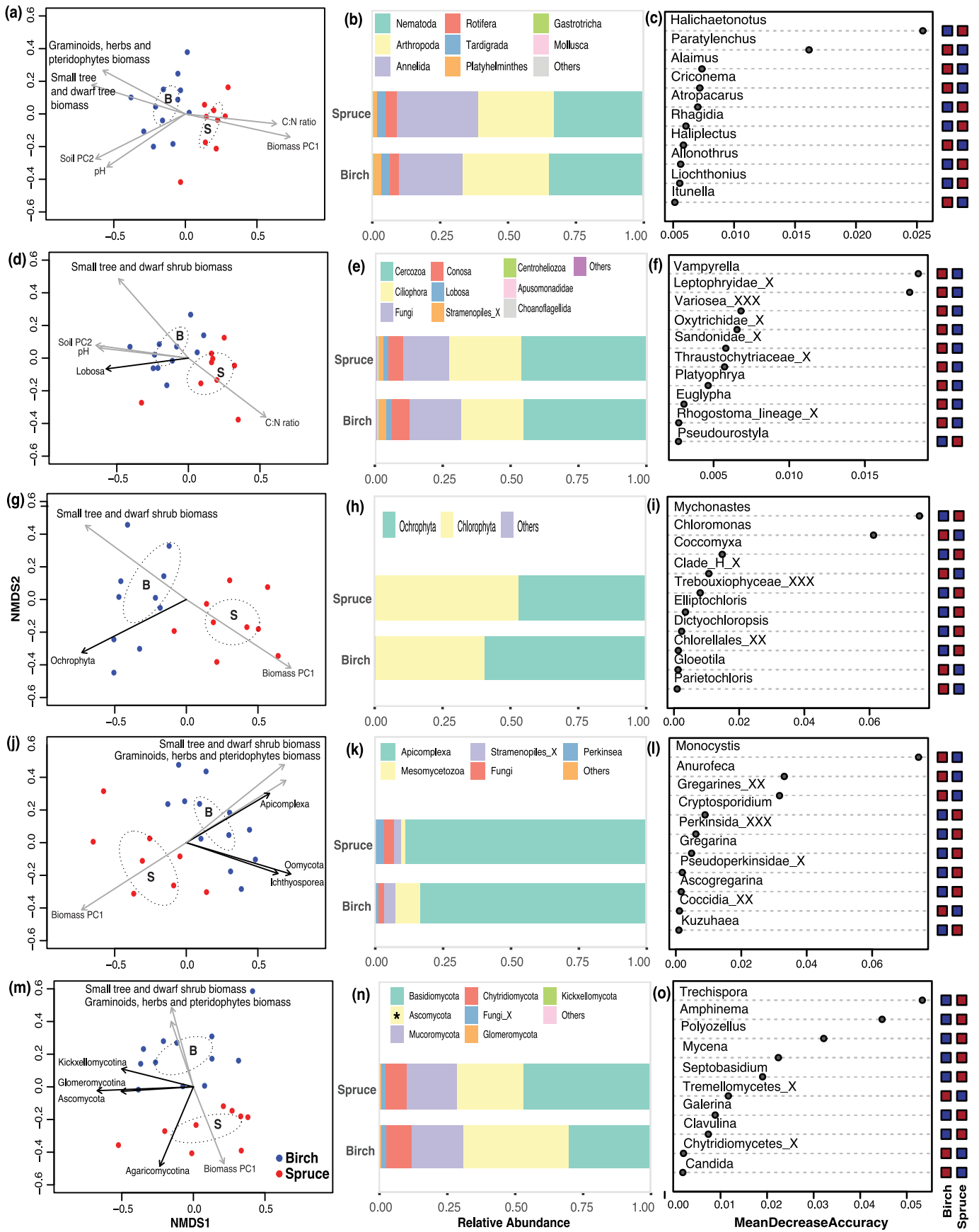
Distinct stand type related parasite communities were largely structured by the understorey vegetation biomass (Fig. 1j); significant soil parameters being limited to S and Mn (Table S3). The lower parasite abundance in spruce may be due to the relatively dense canopy [2] which limits air dispersal of parasitic protists in forest ecosystems [22]. The higher abundance of Mesomycetozoa in birch indicate bottom-up control of the nematodes and arthropods through parasitic gut infection [23] (Fig. 1k). Similarly, we found a higher abundance of Apicomplexa in spruce, mostly animal parasites (*Cryptosporidium*) (Fig. 1l), whose abundance is associated with Arthropoda [24], indicating possible control through infection at higher trophic level.

The osmotrophs communities were mainly fungi that differed distinctly between forest stands, The understorey vegetation biomass was the major structuring factor (Fig. 1m) followed by Co, Si, pH, C, C/N ratio, and Pb (Table S3) [4]. We found higher dominance of basidiomycetes (particularly Agaricomycetes genera *Trechispora*, *Amphinema* and *Mycena*) in the spruce stand (Fig. 1n,o). The *Mycena* genus may play an important role in organic matter turnover through Mn-peroxidase activity in spruce stands [25]. The varying abundance of Mucoromycota, Chytridiomycota, Glomeromycotina and Kickxellomycotina may be ascribed to marker bias (18S vs ITS2).

The abundance of eukaryome at taxonomic and functional category levels differed in birch (e.g., *Alaimus-Vampyrella-Fungi*) and spruce stands (e.g., *Marionina-Pseudourostyla-Mychonastes-Fungi*), indicating different food-webs in operation. Potentially, understorey vegetation-mediated as well as tree litter-mediated changes in soil C and N were driving factors (e.g., bottom-up control through osmotroph-phagotroph-Metazoa). The observed decrease in both phagotrophic and fungal diversity in spruce may suggest a potential relationship. Our findings indicate that tree species and understorey vegetation are important factors for eukaryome community dynamics, and this study shed light on possible food-web mechanisms and controls to be considered for implementing forest management strategies.

## Research data for this article

The fastq-formatted sequence data sets for 18S markers gene, barcode mapping files and linked metadata were deposited at Zenodo (10.5281/zenodo.4 415 050).



(caption on next page)

**Fig. 1. Eukaryome composition analyses displayed by Global Nonmetric Multi-Dimensional Scaling (GNMDS), relative abundance plots and random forest analyses plots.** The sample-based ordination plots for taxonomic group (Metazoa) and functional categories (phagotrophs, phototrophs, parasites and osmotrophs) (a,d,g,j,m). Different tree species are colour-coded (birch = blue and spruce = red). Ellipses are drawn for visualization of the birch and spruce stands and include a 95 % confidence interval of the respective stands. Arrows point in the direction of maximum increase of individual vector variables ('Understorey vegetation biomass index' (biomass PC1), 'Soil chemistry variability index' (soil PC2) and taxa abundance) and had significant effects ( $p < 0.05$ ) on the ordination configuration. Plots showing relative abundance distribution of different taxon (b,e,h,k,n) and rank importance of random forest analyses (c,f,i,l,o) of the taxonomic group (Metazoa) and functional categories (phagotrophs, phototrophs, parasites and osmotrophs). The abundance indicators next to panels (c,f,i,l,o) of the figure indicates the eukaryome OTUs that differ significantly (LEfSe, FDR adjusted  $P < 0.05$ ) between birch and spruce stands (blue = low abundance and red = high abundance). Mean Decrease Accuracy values are calculated based on loss of accuracy while excluding each variable. The significantly different ( $p < 0.05$ , paired T-test) taxa in terms of abundance between stands are indicated using '\*\*' in the figure labels (b,e,h,k,n).

### CRedit authorship contribution statement

**Sunil Mundra:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Dinesh Sanka Loganathachetti:** Visualization, Writing – review & editing. **Håvard Kausserud:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Anna Maria Fiore-Donno:** Data curation, Writing – review & editing. **Tonje Økland:** Methodology, Writing – review & editing. **Jørn-Frode Nordbakken:** Methodology, Writing – review & editing. **O. Janne Kjønaas:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing – review & editing.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejsobi.2023.103593>.

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