

Differences in soil fungal community structure driven by grassland management not sampling period

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Abstract

This study investigated if differences in soil fungal community structure occur between intensively and extensively managed permanent grasslands, and whether they persist across management events and changes in weather conditions within the growing season. Soil samples were taken from both grassland management intensities six times through 2017 (May – October) in the Zurich region, Switzerland. For fungal community structure (measured using Illumina Miseq next generation sequencing), the influence of management ($\sqrt{CV}=0.227$, $P\leq 0.001$) was much greater than that of sampling event ($\sqrt{CV}=0.079$, $P\leq 0.001$). Importantly, no interaction occurred between management and sampling event ($P>0.05$), and consequently, pairwise significant differences in fungal community structure between intensive and extensive grasslands persisted across each of the sampling events (all at least $P\leq 0.05$). These results highlight the temporal stability of pairwise differences in soil fungal structures between contrasting grassland management intensities through the whole growing season, despite changing meteorological conditions and multiple management events.

Keywords: permanent grassland, management intensity, soil fungi, sampling events

Introduction

The soil microbiome is fundamental in a number of agroecosystem processes such as soil nutrient cycling, organic matter decomposition and plant productivity (Bertola *et al.*, 2021). Grassland management intensity is a major driver of grassland soil fungal community structure, with this influence having been observed both at the continental scale and in multiple European regions, as was recently reported in the BIOINVENT project (Barreiro *et al.*, 2022; Fox *et al.*, 2021). Soil microorganisms can, however, also exhibit temporal variation, though the extent to which this shapes the structure of the soil microbial community in agricultural systems remains poorly understood (Dunn *et al.*, 2021). It is an important knowledge gap, as the pertinence of incorporating temporal aspects into soil microbiome studies is being increasingly highlighted in the literature (Geisen, 2021). This is particularly the case with managed grasslands, as not only do climatic conditions and plant phenological stage change as the growing season progresses, but they also undergo numerous management events over this time period (i.e. fertilization, cuttings). The principal aims of this study were to test: (1) if important differences in soil fungal community structure occur between intensively and extensively managed grasslands; and (2) whether these persist throughout the entire growing season.

Materials and methods

Two sharply contrasting grassland management systems were sampled (five fields of each) around the Zurich region of Switzerland. These were: (1) a highly intensively managed permanent grassland (INT, high fertilization with early, frequent utilizations); and (2) an extensively managed permanent grassland (EXT, no fertilization with infrequent utilizations). Soil samples were taken six times throughout the 2017 growing season (May – October), along which management events occurred (i.e. cuttings, fertilizer applications), as well as changing weather conditions. At each sampling, sixteen soil cores were taken along a transect in the field centre, using a soil auger (\varnothing 2.5 cm) to a depth of 20 cm. Soil DNA was extracted from each sample, with the fungal internal transcribed spacer region (ITS2) being PCR

amplified and an amplicon-based Illumina Miseq sequence analysis conducted to generate operational taxonomic units (OTU, 97% sequence identity). A pairwise Bray-Curtis dissimilarity matrix was then constructed, which served as the response in a repeated measures PERMANOVA to determine the influence of grassland management intensity (factor ‘Manage’), sampling event (factor ‘Time’) and the Manage×Time interaction on fungal community structure (PRIMER-7 statistical software package, version 7, Plymouth UK). The variance explained by each factor in the model was reported as the square-root of the component of variation (\sqrt{CV}). A constrained ordination, maximizing the differences within ‘Manage’ and ‘Time’ was conducted in R, using the vegan package for figure construction (R core team, 2021).

Results and discussion

The factor ‘Manage’ had a strong determinant effect on fungal community structure, as indicated by the square-root of the component of variation of the model ($\sqrt{CV}=0.227$, $P\leq 0.001$, Table 1) and evident from Figure 1A. The management effect was far stronger than the ‘Time’ effect ($\sqrt{CV}=0.079$, $P\leq 0.001$). A stronger effect of land-use on soil microbial community structure, compared to sampling event, has been demonstrated previously (e.g. Gschwend *et al.*, 2021), but such studies have used very different land-use types (i.e. grasslands vs forest). Here, we even demonstrate this effect within the same land-use type of permanent grassland, just with contrasting management regimes.

Importantly, there was no significant ‘Manage’×‘Time’ interaction ($P>0.05$, Table 1). Consequently, the clear, highly significant differences in fungal community structure between INT and EXT persisted throughout the six sampling events over/across the growing season (all at least $P\leq 0.05$, analysis not shown). The lesser influence of sampling event is also apparent from Figure 1B. Here, despite using a constrained ordination maximizing the differences between the six sampling events, there is still a clear separation between INT and EXT. Furthermore, little variation in fungal community structure across the six sampling events is evident in each sampling replicate, with the points of the six sampling times

Table 1. Results of a repeated measure PERMANOVA model of the effect of the two grassland management intensities (Manage), sampling event (Time), and the Manage×Time interaction on fungal community structure.¹

Factor	df	MS	Pseudo F	\sqrt{CV}
Manage	1	2.149	3.385***	0.227
Rep(Manage)	8	0.637		0.308
Time	5	0.139	1.783***	0.079
Manage×Time	5	0.083	1.057 ^{ns}	0.030
Residual	39	0.078		0.280

¹ Rep(Manage) denotes the term for the replicate sites over which Manage is tested. Shown are the degrees of freedom (df), the mean sum of squares (MS), the Pseudo-F value and the square-root of the component of variation (\sqrt{CV}). Significance: ‘***’ $P\leq 0.001$, ‘ns’ $P>0.05$.

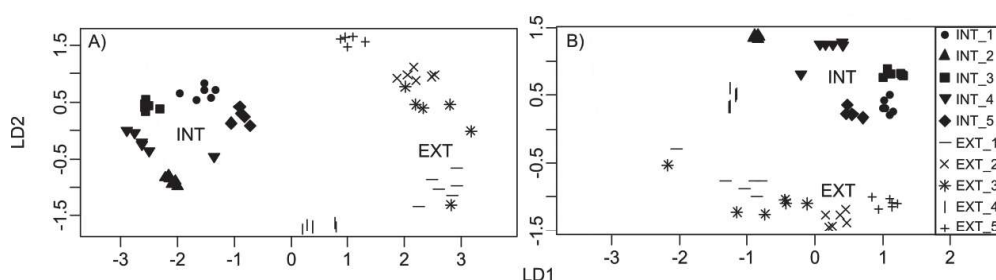


Figure 1. Constrained ordination (based on canonical analysis of principal coordinates) of fungal community structure (A) maximizing the differences between the five intensively (INT, filled symbols) and extensively (EXT, line symbols) managed grassland sites across the six sampling events and (B) maximizing the differences between each of the six sampling events across the two management intensities.

clustering tightly together (Figure 1B). Recently, the BIOINVENT project demonstrated the strong influence of grassland management (i.e. intensive vs extensive) on soil fungal community structure in multiple European regions (Fox *et al.*, 2021), though this study only utilized a single sampling event at the peak plant productivity of the growing season. The results presented here both confirm and strengthen this finding, and demonstrate that such management-induced differences are not merely present at peak plant productivity, but persist regardless of plant phenological stage or individual management events.

Conclusions

Our study highlights that sharp differences in fungal community structure are induced by grassland management. These differences are temporally stable throughout the entire growing season. These results strengthen the findings reported in the BIOINVENT project, which also demonstrated the strong influence of grassland management on soil fungal community structure in multiple European regions, though it utilized only one sampling event.

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