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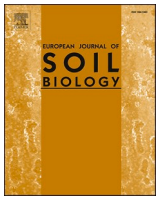

Original article

# Impact of grassland management intensity on associations between bacterial, fungal and plant communities

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ABSTRACT

Understanding co-occurrences of different taxa is of both fundamental and applied relevance, for example, to understand ecosystem processes and to design monitoring programs for above- and belowground biodiversity. Plants and microorganisms form complex, interdependent relationships, which are exposed to and may be compromised by agricultural management. Here we assessed the effect of grassland management intensities on bacterial, fungal and plant communities and their associations. We further analyzed the potential of inferring information from taxa of one community on structural changes of the other communities with the aim of potentially enhancing the efficiency of biodiversity assessments by finding common indicator taxa. For that, bacterial, fungal and plant communities as well as environmental factors were assessed in 89 grassland sites of either extensive type (no fertilization, late and infrequent cuttings) or intensive type (fertilization, early and frequent cuttings) of management in the Swiss lowlands.

Bacterial, fungal and plant community structures as well as plant indicator values for soil nutrients and moisture differed between management types. Also, community homogeneity was significantly higher for all communities in the intensively managed grassland. For bacterial community structures, this was likely related to a smaller soil pH range in intensively managed grassland, while a lower fungal and plant richness may have caused more homogenous fungal and plant community structures in intensively managed grassland. Further, correlation strength among community structures dropped by 25–66 % from extensively to intensively managed grassland. Finally, indicator analysis suggested that future monitoring programs may use plant taxa to estimate expected effects on fungal communities and vice versa, but bacterial communities require additional assessment. Our results revealed a multifaceted and profound effect of management on bacterial, fungal and plant communities, which reinforces the conservation value of extensively managed grassland.

## 1. Introduction

Land-use intensification of grasslands in terms of fertilizer input, cutting and grazing intensity leads to changes in the above- and belowground communities which in turn may affect associations between biotic and abiotic components of the habitat [1]. Between the 1960s and the 2010s, grassland intensification led to a plant species loss of 30–50 %. This was accompanied by a shift towards nutrient-demanding, competitive species and a decrease of early-flowering species and insect-pollinated herbs in grasslands of Northern Germany [2]. Also, fertile permanent grasslands in three different regions of Switzerland experienced a shift in plant species composition to species characterized by high competitiveness under high nutrient conditions, high forage quality and tolerance to mowing

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from the 1970s–2000s [3] These changes are important at a large scale, as about 34 % of the European Union's agricultural area is covered by permanent grassland and may experience intensification [4]. Therefore, the biodiversity of extensively managed grassland, i.e., unfertilized grassland with late cuttings, is under threat and a focus on biodiversity protection strategies is urgently needed.

Land-use intensification affects not only plant communities but several abiotic and biotic components in above- and belowground habitats of grassland ecosystems. This was, for instance, revealed in an assessment of 14 guilds of organisms, which covered primary (e.g., protists), secondary (e.g., arthropods) and tertiary (e.g., birds) consumers in the terrestrial food web, primary producers (plants), and decomposers (e.g., fungi), where guilds were selected based on their proposed nutrient acquisition strategies, i.e., from slow to fast [5]. In this study, most guilds along the gradient of slow to fast growers followed a gradient from low to high fertilization as well as cutting and grazing frequency, supporting the hypothesis that extensively managed grasslands harbor rather slow-growing, while intensively managed grasslands are dominated by fast-growing organisms [5]. Furthermore, land-use intensification can lead to more homogenous above- and belowground communities [6]. This homogenization effect may be due to the dispersal of a few generalist species and/or the loss of rare and specialized species [6]. In addition, extensively managed grasslands may harbor more dissimilar communities because these grasslands are often situated on less fertile soils or difficult to manage areas creating habitat conditions for specialized communities [7]. Intensification of grasslands may have a number of different effects on communities including direct disturbances such as cutting or grazing, the addition of nutrients and slowly degrading structures (e.g., pieces of straw) as habitats, e.g., by the application of organic or inorganic fertilizer [8,9]. Further indirect effects include changes of soil conditions such as pH [10], and interactions among organisms, e.g., of different trophic levels.

Above- and belowground species communities form intimate relationships [11]. For instance, plants as primary producers sequester carbon and provide nutrients to soil communities via root exudates or dead plant material for consumption. Further, plant species provide habitats and niches for other organisms. In return, soil communities as decomposers and symbionts provide nutrients to the plants, for instance, by fixing nitrogen or converting organic matter to plant-available forms of nitrogen [12]. The symbiotic relationships are manifold and range from mutualism to parasitism [13]. Studies on the effect of land-use intensity on above- and belowground communities in grassland suggest that they respond rather differently to the same disturbance, i.e., land-use intensity. It has been shown that species richness of aboveground trophic groups was strongly negatively associated to land-use intensity in grassland while species richness of belowground trophic groups was positively or not associated at all [1]. In an experiment with different mixtures of grasses and legumes, intense mowing affected plant coverage as well as bumble bee species richness and abundance positively and earthworm richness and biomass negatively [14]. Also, plant and soil microbial communities differed in their resistance and resilience to N fertilization and mowing in Eurasian steppe [15]. Nevertheless, even if general measures of biodiversity, such as species richness, abundance and biomass of above- and belowground communities differ in their response to disturbance, certain components of the communities might respond in the same way. Then, it would be sufficient to assess responses of one type or components of one type of community and from that predict the responses of other communities. This would reduce the efforts of assessment and monitoring of biodiversity and would facilitate the search for strategies for protecting biodiversity.

Here we jointly analyze data from two independent grassland studies in Switzerland, i.e., "BIOINVENT" (BI) [16] and "SOILSERVICEGRASS" (SSG) [17,18], in which soil bacterial and fungal communities were assessed across extensively and intensively managed grasslands using metabarcoding in a comparable manner (see Supplemental file 1). In BI, bacterial and fungal communities were assessed at sites with different grassland management intensities in regions with conditions of higher or lower potential for plant biomass production [16]. In the study SSG the effects of management intensities, harvest types, i.e., mostly grazing or mowing and management practice, i.e., organic vs conventional farming, on the prokaryotic (bacterial and archaeal) and fungal grassland communities were assessed [17]. Plant species and cover were obtained in both studies in detail but were not yet published (BI) or only in a summarized manner (SSG). The present study aims at obtaining a more holistic view by integrating above- and belowground communities and to better understand how grassland management intensity influences the relationship of plant communities with soil bacteria and fungi by combining both studies. Specifically, we hypothesize that:

1) The soil bacterial and fungal as well as plant communities of intensively managed grasslands will be more homogeneous than those of extensively managed grasslands. In line with this, we postulate that the correlations between these different community structures (β-diversity) will be weaker in intensively than in extensively managed grassland.
2) Specific components of one community (bacterial and fungal amplicon sequencing variants as well as plant species) will be correlated with the community structures of the other communities. The existence of such associations will allow the identification of suitable indicators among taxa of multiple types of organisms.

## 2. Material and methods

### 2.1. Sites and management factors

In total, 89 grassland sites were selected from two studies, i.e., "BIOINVENT" (BI), from which 12 extensively and 12 intensively managed sites in the lowland of Switzerland [16] and "SOILSERVICEGRASS" (SSG), from which 35 extensively and 30 intensively managed sites were chosen [17,18]. From the latter project, only intensively managed sites which were fertilized with more than 60 kg of available nitrogen $ha^{-1}$ $y^{-1}$, were included. Detailed descriptions of the methods can be found in the respective publications [16–18]. Here, we summarize the main parts of the sampling and analytical procedures, the points in which the projects differ, and add information that is specific for the present study only. The sites were spread across the cantons of Solothurn (only SSG), Aargau, Zürich and Thurgau (all BI), which are part of the Swiss Central Plateau and cover an area of 4915 $km^2$. The average parcel size of permanent grassland in the canton of Solothurn was 0.9 ha in 2023 [19]. All sites were part of farms that were eligible for direct payments based on the Swiss eco-scheme of ecological focus areas for which a minimum of 7 % of the agricultural area needs to be dedicated as ecological focus area (Swiss direct payment regulation SR910.13). Further, sites were chosen with the aim of including a large gradient of management intensities and obtaining two groups of sites of equal size with clearly distinct management intensity. Sites that were managed according to the Swiss eco-scheme "extensively managed meadows" and "extensively managed pastures" and thus belonging to the ecological focus area of the farms (not fertilized and, in the case of meadows, receiving a late first cut after June 15th) were defined as an extensively managed grassland, while sites not managed according to these Eco-schemes and which were fertilized with more than 60 kg of available nitrogen $ha^{-1}$ $y^{-1}$ were defined as intensively managed grasslands. The Swiss eco-scheme enabled the inclusion of extensively and intensively managed sites mostly from the same farm unit to avoid potential bias due to geographic factors such as elevation. Management intensity was further specified by the level of applied available nitrogen, cutting frequency, number of grazing days and grazing intensity in livestock units (LSU) $ha^{-1}$ $y^{-1}$. This information was retrieved directly from the farmers via questionnaires and the mean and standard deviation of each factor are presented in Table S1.

### 2.2. Sampling and soil factors

At each site, 16 (BI) or 20 (SSG) soil cores with a diameter of 1.4 (SSG) or 2.5 cm (BI) and 20 cm depth were sampled from an area of 20 m × 20 m and combined. The soil cores were sampled along two straight lines that formed a cross within the 20 m × 20 m area. Soil samples were passed through a 2 mm sieve and homogenized. Of the soil and geographic factors that were assessed in both studies, the following were measured with compatible methods: total nitrogen by combustion, pH in water extract, texture as percent clay, sand and silt, and elevation (Table S1). Organic carbon was unfortunately measured with different methods; therefore, we were reluctant to include it in a combined analysis. However, we assessed the difference of organic carbon-to-nitrogen ratio between management types within each study. For detailed descriptions of the methods employed see [16–18].

### 2.3. Plants and plant derived indicator values

All vascular plant species were determined, and their coverages were estimated in two (SSG) or four (BI) quadrats with a size of 2 m × 2 m each. The quadrats were situated within the 20 m × 20 m area from which soil samples were obtained. For both the SSG and BI sites, the cover was directly noted in steps of one percent. For the sites of BI, two of the four quadrats were randomly chosen to obtain the same number of quadrats per site in both studies (same sampling effort).

The overall species richness of both quadrats and the mean value of the cover were used for further analyses. Habitat preferences of plant species can be exploited to infer information on soil conditions from the presence of certain plants [20]. For that, each plant species was evaluated and assigned with indicator values, which were defined by Landolt et al. for the flora of Switzerland and the Alps [20]. Cover-weighted plant indicator values for soil nutrients and moisture were obtained for each quadrat and mean indicator values for soil nutrients (pi-N) and for soil moisture (pi-M) were calculated (Table S1).

### 2.4. Bacterial and fungal communities

Metabarcoding of 16S v3-4 and the ITS2 region was used to assess bacterial and fungal communities. For that, total DNA was extracted from 0.25 (SSG) or 0.3 g (BI) of mixed and sieved soils using DNeasy PowerSoil Pro QIAcube Kit (Qiagen, Germany) in the SSG project or a phenol-chloroform extraction according to the procedure previously described and modified [21,22] in the BI project. PCR was performed in quadruplets for each sample, which were then pooled, using the same primer pair for bacteria (341F and 806R; modified by Frey et al., 2016 [23]). For fungi the primer pair ITS3/ITS4 was used, however, with slightly different nucleotide variations, i.e., in the SSG project, it contained five more ambiguous positions to potentially increase coverage of fungal taxa [24] compared to the BI project [25] (Supplemental file 1, Table S2). Comparison of primer sequences and potential effects on downstream analyses are discussed in Supplemental file 1. The analysis led to the conclusion that the data of the two projects are compatible. PCR libraries were prepared using the Fluidigm Access Array System (Fluidigm, U.S.A.) and sequenced with a paired end 300 run and the Illumina MiSeq v3 platform (Illumina Inc., U.S.A.) at the Functional Genomic Center Zurich (SSG; Switzerland) and the Génome Québec Innovation Center in Montréal (BI; Canada).

### 2.5. Bioinformatic analyses

Raw sequences are available at the NCBI SRA archive under the project number PRJNA641340 for BI and in the European Nucleotide Archive under the accession number PRJEB72428 for SSG. Quality control of the raw sequences and taxonomic classification was performed using a pipeline mostly based on the software "vsearch" version 2.27.0 [26] and "mothur" version 1.47.0 [27] according to an already published pipeline [23,28]. In short, spiked phiX sequences were removed using "bowtie2" [29]. Primers were removed using "cutadapt" version 3.5 [30] and these first two step were performed separately for fungal sequences from BI and SSG with the respective primer sequences. For all the following steps the sequences of both projects were combined. The software "vsearch" was used to merge paired end sequences, remove sequences with high error rates and dereplicate sequences prior to "de-noising" to obtain amplicon sequence variants (ASVs) using the "unoise" algorithm. Potentially chimeric ASVs and ASVs without organism-specific patterns were removed using the "uchime3_denovo" algorithm and "metaxa2" version 2.2.3 for bacterial or "ITSx" version 1.1.3 for fungal ASVs [31,32]. Taxonomic classification was performed with Bayesian classifier using "mothur" and for bacterial and fungal ASVs the GTDB ssu release 214 [33] and UNITE version 9.0 databases [34]. The GTDB was downloaded in March 2024 and the following steps were used to curate and adapt the database. First, sequences were dereplicated by taxonomy in chunks of 50,000 sequences using "MetaCurator" [35]. Afterwards sequences were filtered for 16S signature using "metaxa2". The GTDB does not contain sequences of plant organelles, i.e., chloroplasts and mitochondria, which need to be identified and excluded from the data. Therefore, sequences of the GDTB that matched with more than 97 % identity to sequences of plant organelles from the SILVA database release 138.1 [36] were removed using "vsearch" and organelle sequences SILVA were then added to the GTDB. Finally, the database was filtered for sequences between 500 and 2000 nucleotides. Curation reduced the database from 708,813 to 154,705 sequences. ASVs that were not classified as bacteria (organelles and archaea), or fungi (e.g., protists and plants), or were unclassified at the domain-level for bacteria and at the kingdom-level for fungi were removed. Primary and secondary fungal traits based on genera were obtained from the "FungalTraits" database [37].

### 2.6. Statistical analyses

Statistical analyses were performed in R version 4.2.3 within RStudio [38]. Bacterial and fungal ASV abundances were subsampled to the lowest number of sequences of a sample using the function "rrarefy" of the R-package "vegan" [39] and this process was iterated 100 times [40]. ASV richness and Bray-Curtis dissimilarities were calculated for each of the 100 subsampled ASV tables using the functions "specnumber" and "vegdist" ("vegan") and the median of the 100 iterations was calculated. Although sequencing depth differed significantly between the two projects, it did not affect ASV richness confirming the compatibility of the two projects (Supplemental file 1, Fig. S2). For plant community structures, Bray-Curtis dissimilarities were calculated from relative coverage. Soil and geographic factors, i.e., pH, total nitrogen, clay, sand, silt, elevation and pi-N (omitting pi-M because it was strongly correlated with pi-N, i.e., rho = 0.89 and p-value <0.001) were centered and scaled before Euclidean distances were calculated. Differences in community structures and combined environmental factors between management types were assessed with PERMANOVA using the function "adonis2" in the R-package "vegan". Pairwise Pearson correlations between community structures as well as with Euclidian distance of each environmental factor were calculated within extensively (47 sites) and intensively (42 sites) managed grassland using the function "mantel" ("vegan"). Partial Mantel tests were used to condition for pH using Euclidian distances of soil pH using "mantel.partial" ("vegan"). P-values in PERMANOVA and Mantel tests were based on 1000 permutations. Dispersion of each community and environmental factors was determined by calculating distances to centroids of each management type using the function "betadisper" ("vegan"). Differences of distances to centroids between management types were tested using the function "permutest" ("vegan") with 1000 permutations. Unconstrained ordinations were calculated using the function "metaMDS" ("vegan"). Differences of univariate variables, i.e., richness, soil and geographic factors, management related variables among factors (management types,

clusters, which are defined in the text below, and projects) were assessed using Kruskal-Wallis rank sum test followed by Dunn's Kruskal-Wallis Multiple Comparisons (function "dunnTest" in R-package "FSA" [41]). P-values were adjusted using Benjamini-Hochberg p-value correction (BH, [42]) with the function "p.adjust". Hierarchical clustering and k-means clustering were based on Bray-Curtis dissimilarities and explained in detail in Supplemental file 2. Indicator species analyses were performed using the function "multipatt" in the R-package "indicspecies" [43] using the "Indval.g" statistic and only taxa, i.e., bacterial and fungal ASVs as well as genera, plant species and fungal primary as well as secondary traits, with a value above 0.8 and a significant BH-adjusted p-value ($p \leq 0.05$) were defined as strong indicators.

## 3. Results

### 3.1. Effect of grassland management on community structures

Bacterial, fungal and plant community structures differed significantly between extensive and intensively managed grassland (Fig. 1; Table 1). The strongest effect of management intensity was observed for plant community structures (distance between centroids of 0.549; $R^2 =$ 23.7 %), followed by fungi (0.319; 8.5 %) and bacteria (0.199; 4.4 %). Of the variables directly related to management, available nitrogen from fertilizer, grazing intensity, and cutting frequency were also significantly higher in intensively managed grassland, while number of grazing days did not differ (Fig. S6). The combination of environmental factors, i.e., soil pH, total nitrogen, silt, clay, elevation and the mean indicator value for soil nutrients (pi-N), also differed significantly between the two management types (Table 1). Importantly, this difference depended strongly on pi-N, because after removing it from the dataset, no significant difference of the remaining factors between management types was observed anymore. The median pi-N was significantly lower (2.96) in extensively than in intensively managed grassland (3.82), suggesting higher nutrient content in soils with intensive management (Fig. S7). Also, the mean indicator value for soil moisture (pi-M), which was strongly positively correlated with pi-N (rho = 0.89, $p < 0.001$), was significantly higher in intensively managed grassland (3.21 versus 2.79). The difference of community structures between management types may also be driven by differences in soil organic carbon-to-nitrogen ratio. We were reluctant to include the factor in the overall analysis, because organic carbon was measured with different methods in the two studies. However, separate analyses for each study showed no significant difference between management types for BI and very small differences for SSG (Fig. S8). Except for pi-N and pi-M, none of the soil and geographic factors but most of the management related factors differed between management intensities, suggesting that management intensity was the main driver of the differences in community structures between management types (Fig. S7).

We found more homogenous communities in intensively managed grassland for all three community types (Fig. 1; Table 1). This was represented by a lower dispersion of the community structure, measured as the average distance of each community to the centroid of the community, in intensively compared to extensively managed grassland. The strongest effect of management was detected for fungi and plants, for which the dispersion was 16.8 and 15.3 % higher in extensively than in intensively managed grassland. This was followed by bacteria (9.1 %), and no significant effect was observed for soil and geographic factors (Fig. 1; Table 1). Significant PERMANOVA results (Table 1) may originate from different distances between group centroids or differences in dispersion. The ordinations clearly reveal distinct communities of extensively and intensively managed grasslands, that differ in both the distance between centroids and the dispersion especially for fungal and plant communities (Fig. 1).

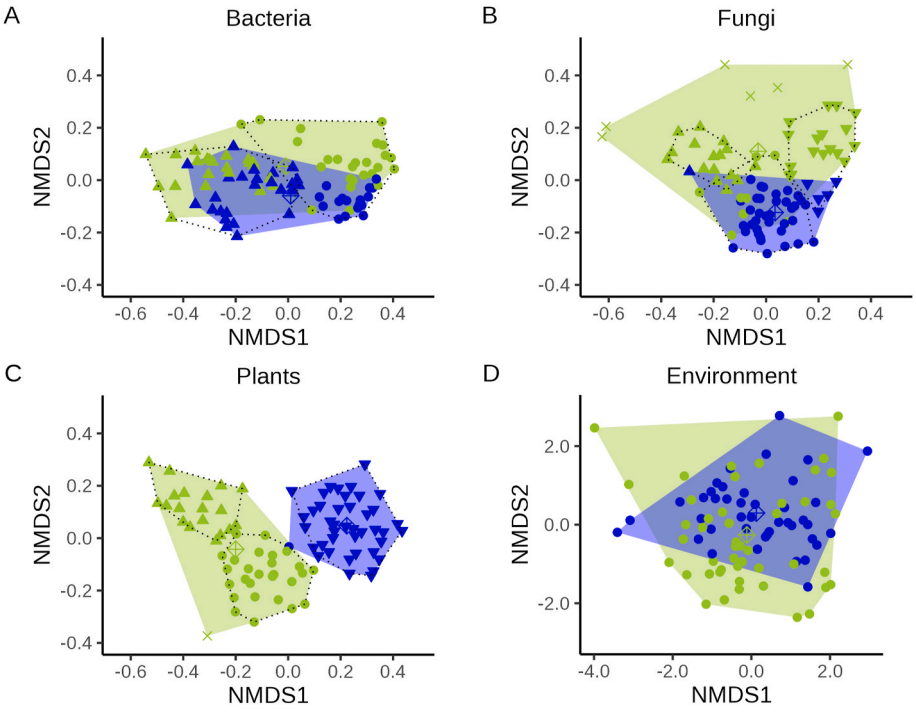


**Fig. 1. Community structures and environmental factors between management types as well as clusters:** Non-metric multi-dimensional scaling (NMDS) ordination displaying bacterial (A), fungal (B), plant (C) community structures as well as soil and geographic factors including pi-N (D) between management types and clusters. Green shapes and areas represent extensively (unfertilized and late cutting, i.e., first cut after June 15th) and blue shapes and areas intensively (fertilized and early cutting allowed) managed grassland. Upward triangle, circle, downward triangle represent cluster 1, 2 and 3, respectively. The crossed squares indicate centroids for each management type and the x display outliers in the cluster analysis. Dotted black lines indicate the area of each cluster's sites. Ordination stress values in panel A, B, C and D were 0.10, 0.15, 0.16 and 0.19. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**
Differences in community structures and dispersion of community structures of bacteria, fungi and plants (Bray-Curtis) as well as distances (Euclidean) based on soil and geographic factors between extensively and intensively managed grassland.

| | PERMANOVA | | | | PERMDISP | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Distance between centroids | $R^2$ [%] | F | p-value | Median distance to centroid | | Difference in dispersion between extensive and intensive [%] | F | p-value |
| | | | | | Extensive | Intensive | | | |
| Bacteria[a] | 0.199 | 4.4 | 4.0 | 0.003 | 0.478 | 0.438 | 9.1 | 8.7 | 0.003 |
| Fungi[a] | 0.319 | 8.5 | 8.1 | 0.001 | 0.555 | 0.475 | 16.8 | 43.8 | 0.001 |
| Plants[a] | 0.549 | 23.7 | 27.0 | 0.001 | 0.511 | 0.443 | 15.3 | 18.7 | 0.001 |
| Soil & geographic factors incl. pi-N[b] | 1.705 | 12.2 | 12.1 | 0.001 | 2.274 | 2.004 | 13.5 | 2.7 | 0.112 |
| Soil & geographic factors excl. pi-N[b] | 0.600 | 1.8 | 1.6 | 0.166 | 2.129 | 1.982 | 7.4 | 0.8 | 0.376 |

[a] Bray-Curtis dissimilarity.
[b] Euclidean distance.

Soil pH can influence community structures and, indeed, although the mean did not differ significantly between the management types, the pH range was larger for extensively managed grasslands, i.e., 5.2 to 8.0 compared to 6.0 to 8.0 for intensively managed grassland (Fig. S7). Therefore, we tested whether community structures and homogeneity of community structures differed among management types with a reduced dataset of the same pH range, i.e., 6 to 8, for both management types. This selection resulted in a dataset of 74 sites with 32 extensively and 42 intensively managed grassland sites. Differences of structures of all three communities between management types were very similar to those observed in the entire dataset of 89 sites (Table 1 and S4). Also, dispersion of fungal and plant communities was comparable in both datasets (Table 1 and S4). In contrast, dispersion of bacterial community structures did not differ significantly between management types anymore, when using sites within the same pH range. Therefore, the larger heterogeneity of bacterial communities in extensively managed grassland may be attributed to a larger pH range in extensively than in intensively managed grassland. In addition, one possible reason for an increased homogeneity in intensively managed grassland is the loss of taxa. Indeed, fungal ASV and plant species richness were significantly lower in intensively managed grassland, suggesting that an increased homogeneity may be connected to a loss of taxa (Fig. 2). In contrast, bacterial ASV richness was even significantly higher in intensively managed grassland. In summary, independent of the measured soil and geographic factors, intensive management led to more homogenous fungal and plant communities with lower species/ASV richness than extensively managed grassland. For bacterial communities, the greater heterogeneity in extensively managed grassland was most likely due to a larger pH gradient.

### 3.2. Comparison of communities across and within extensive and intensive grassland

The assessment of correlations among communities, which could be interpreted as similar habitat preferences or potential interactions, revealed that all pairwise correlations of community structures of different organism types were significant across and within both management types (Table 2). The strongest correlations were detected between bacterial and fungal community structures across and within management types (r between 0.61 and 0.82). Correlations between community structures of bacteria or fungi with plants yielded a correlation coefficient of 0.27 and 0.53 overall, of 0.38 and 0.53 for extensively but only 0.14 and 0.18 for intensively managed grasslands, respectively. In other words, a drop of the correlation strength by 25, 62 and 66 % was detected from extensively to intensively managed grassland for the correlations between bacterial and fungal, bacterial and plant as well as fungal and plant community structures, respectively.

After conditioning on soil pH, which was the most influential environmental factor (Table S5), correlations between pairwise comparisons of community types across and within management type were still significant and followed similar patterns as without conditioning for pH, i.e., stronger correlations were found in extensively than in intensively

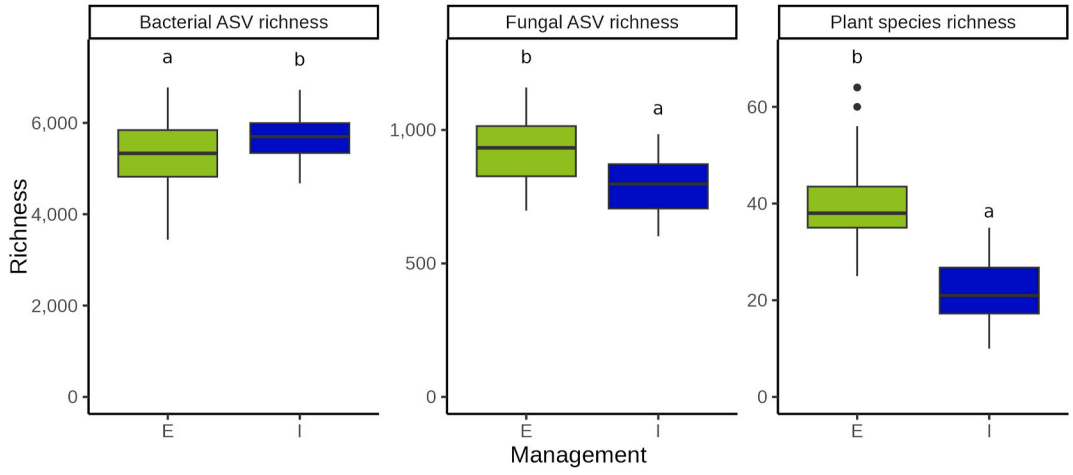


**Fig. 2. ASV and species richness between management types**: Richness of bacteria, fungi and plants in extensively (E) or intensively (I) managed grassland. The bold horizontal line in the boxplot displays the median, the upper/lower limit of the box show the 25th/75th percentile, the smallest/largest values of the whiskers represent 1.5 × the interquartile range of the 25th/75th percentile and outliers are displayed as dots. Letters indicate significant differences based on a p-value ≤ 0.05 using Kruskal-Wallis rank sum test.

**Table 2**
Pairwise correlation between community structures of bacteria, fungi and plants as well as soil and geographic factors in extensively and intensively managed grassland (Mantel test with Pearson correlation). Communities were represented by Bray-Curtis dissimilarities and conditioning on pH was performed with partial Mantel test using Euclidean distances of soil pH.

| Management | Types of organisms | Conditioned on soil pH | Mantel (r) | BH adjusted p-value |
|---|---|---|---|---|
| Extensive | Bacteria & Fungi | No | 0.815 | 0.001 |
| | Bacteria & Plants | No | 0.376 | 0.001 |
| | Fungi & Plants | No | 0.529 | 0.001 |
| | Bacteria & Fungi | Yes | 0.693 | 0.002 |
| | Bacteria & Plants | Yes | 0.230 | 0.002 |
| | Fungi & Plants | Yes | 0.454 | 0.002 |
| Intensive | Bacteria & Fungi | No | 0.610 | 0.002 |
| | Bacteria & Plants | No | 0.143 | 0.003 |
| | Fungi & Plants | No | 0.181 | 0.005 |
| | Bacteria & Fungi | Yes | 0.601 | 0.002 |
| | Bacteria & Plants | Yes | 0.090 | 0.035 |
| | Fungi & Plants | Yes | 0.157 | 0.004 |
| All | Bacteria & Fungi | No | 0.684 | 0.001 |
| | Bacteria & Plants | No | 0.271 | 0.001 |
| | Fungi & Plants | No | 0.527 | 0.001 |
| | Bacteria & Fungi | Yes | 0.559 | 0.001 |
| | Bacteria & Plants | Yes | 0.211 | 0.001 |
| | Fungi & Plants | Yes | 0.513 | 0.001 |

managed grasslands (Table 2). The difference in correlation strength detected in extensively, in comparison to intensively, managed grassland was still between 13 and 65 %. In summary, all correlations were weaker in intensively than in extensively managed grassland and belowground communities (bacteria and fungi) were most strongly correlated with each other, even after taking pH into account. Plant community structures correlated more with fungal than bacterial community structures.

### 3.3. Ecological context of community clusters

Sites with a similar community structure of bacteria, fungi or plants, were clustered and the ecological context of the resulting community clusters was assessed to evaluate the communities without preconceived information, i.e., management types. The clustering results and decisions are described in Supplemental file 2. After removing outliers, i.e., clusters that contained only one or two sites, the best fitting number of clusters was two (bacteria B1-B2) or three (fungi F1-F3 and plants P1-P3), respectively (Fig. 1). Bacterial clusters contained rather similar numbers of extensively and intensively managed grasslands (Table S6) and none of the management related variables differed among bacterial clusters (Fig. S9). Regarding soil and geographic factors, B1 was characterized by a significantly lower pH and total nitrogen content as well as higher pi-N and pi-M values compared to B2 (Fig. S10).

Fungal clusters included sites with a large fraction of certain management intensities, i.e., cluster F1 and F3 contained mostly extensively managed grassland, whereas cluster F2 was characterized by mostly intensively managed sites (Table S6). In accordance, applied available nitrogen, number of grazing days and grazing intensity as well as pi-N and pi-M were significantly the highest in F2 (Figs. S9 and S10). However, number of grazing days and grazing intensity was similarly high in clusters F1 (mostly extensive) and F2 (mostly intensive). Further, soil pH displayed an increasing gradient from cluster F1 to F3 and elevation was significantly lower in F3 than in F1 and F2. The cluster analysis revealed a cluster of mostly extensive management with higher mean number of grazing days and grazing intensity as well as lower soil pH at higher elevations (F1) and cluster of extensive management with the opposite conditions (F3).

The plant clusters mirrored the management types even more strongly (Fig. 1). Clusters P1 and P2 consisted mostly of extensively managed sites, whereas cluster P3 contained intensively managed sites (Fig. 1, Table S6). Similar to the fungal clusters, plant communities also displayed clusters with extensively managed grasslands of either low (P2) or high (P1) pH, whereas P2 had a grazing intensity more similar to intensively managed grassland (Figs. S9 and S10). Furthermore, cluster P2 and F1 shared a substantial number of sites, i.e., 55.6 % of the sites of P2 were also included in F1 and 88.2 % of the sites of F1 were also part of P2.

In summary, cluster assessment corroborated the importance of soil factors for bacterial and in addition grassland management for the fungal and plant communities and revealed that clusters F1 and P2 may represent clusters of sites with more intensively managed extensive grasslands with lower soil pH.

### 3.4. Indicators for community clusters

Indicator species analysis was used to assess how well shifts in belowground community structures may be estimated with measures of the aboveground community and vice versa. For that, the number and identity of bacterial and fungal ASVs and genera, fungal primary and secondary traits as well as plant species that indicate a difference in community structure (represented by different community clusters) were identified. For the indicator species analyses, only taxa were included that occurred in at least ten sites of a cluster (Table S7).

The highest number of strong (Indval.g $> 0.8$) indicators was found for the fungal clusters (Fig. 3, Table 3). In total, 16.1 % of the bacterial ASVs, 13.7 % of the bacterial genera and 10.5 % of the plant species were associated to one or two fungal clusters. The plant species included the grass *Poa trivialis* s.l., which was indicative for the combination of F1 and F2, the grass *Poa pratensis*, which was an indicator for the combination of F2 and F3 as well as the legume *Trifolium pratense* s.l. And the forb *Veronica chamaedrys*, which were both associated to the combination of cluster F1 and F3 containing mostly extensively managed grassland. These plant species represent potential aboveground taxa indicating different fungal community clusters. There were numerous bacterial indicator ASVs and genera for all combinations of fungal clusters, except for F2 and the combination F1 and F3. The latter combination contained sites from both ends of the pH range (Fig. S10B) and the absence of bacterial indicators for this combination suggested a specialization of bacterial taxa for either low or high soil pH.

In comparison to the fungal clusters, only few strong indicator taxa were detected for clusters of bacterial communities, i.e., 3.5 % of the fungal genera, 3.2 % of the fungal ASVs and none of the plant species (Fig. 3, Table 3), revealing that no aboveground indicators were found for bacterial community clusters.

Below-ground indicator taxa were found for all plant clusters and combinations of plant clusters. They included 12.4 % of the fungal genera, 6.6 % of the fungal ASVs as well as two primary fungal traits, i.e., ectomycorrhiza and lichen parasites, which were associated to the combination of P1 and P2 (both mostly extensively managed), and two secondary fungal traits, i.e., ericoid mycorrhiza and root endophytes with dark septate hyphae, which were associated to a combination of P1 and P2 and only P1, respectively. Also, 1.4 % of the bacterial ASVs and 0.2 % of the bacterial genera were associated to plant clusters or cluster combinations.

Elucidating the strongest associations, the 15 strongest indicator taxa for clusters of each type of organism were assessed and the index of

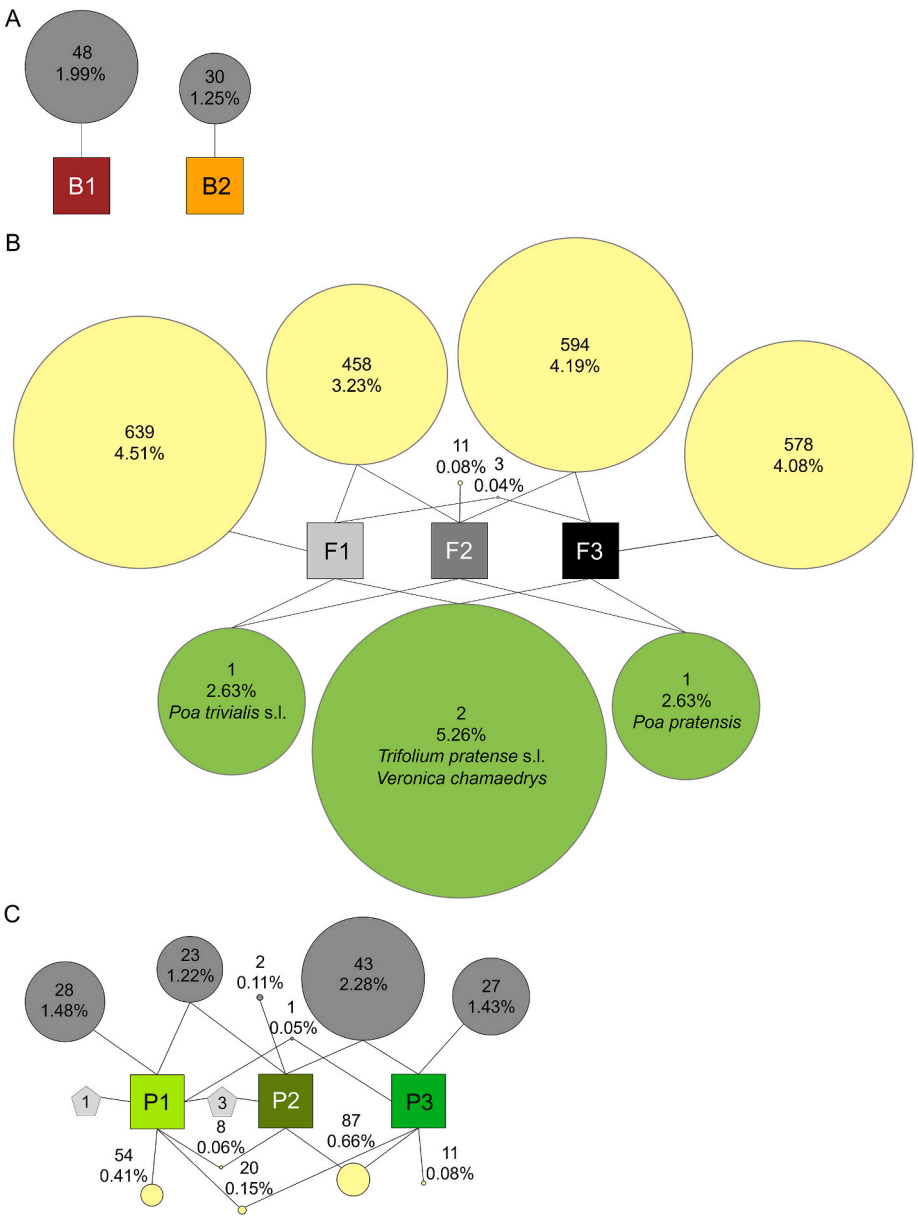


**Fig. 3. Indicator taxa for clusters**: Number of indicator taxa for bacterial (A), fungal (B) and plant (C) clusters. Clusters are shown as colored squares. Grey disks represent fungal indicator ASVs, yellow disks bacterial indicator ASVs and green disks plant indicator species. Light grey pentagons represent fungal primary and secondary traits. Upper and lower number associated to a disk are number and percentage of all taxa that occurred in at least ten sites of a cluster. Radius of the disks corresponds to the percentages of the taxa included in the indicator species analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

association (Indval.g) ranged between 0.919 and 0.996. All indicators for bacterial and plant clusters were fungal taxa and all indicators for fungal clusters were bacterial taxa (Table S8). The strongest indicator taxa for a bacterial cluster were fASV_193 (best classification to order Pleosporales), fASV_56 (classified as *Solicoccozyma terricola*) and fASV_49 (*Saitozyma podzolica*), which were associated to cluster B1, which was the cluster with lower pH and total N content and higher pi-N and pi-M (Table S8). The strongest indicators for fungal clusters were associated to a combination of F2 and F3 and they were the bacterial genus *Lacipirellula*, the bacterial genus V1-33 (order Verrucomicrobiales) and bASV_479 (classification to order Rhizobiales). For plant clusters, the strongest indicators were the fungal genera *Hygrocybe* and *Clavaria*, which were associated to the combination of P1 and P2 and fASV_951 (classified to *Cyphellophora livistonae*) associated to P1 representing extensively managed grassland. Interestingly, among the 15 strongest indicators were associations of potentially plant pathogenic fungi to the combination of P2 (intensively managed grassland) and P3 (extensively managed grassland with elevated grazing intensity and lower soil pH), i.e., fASV_16 classified as *Fusarium graminearum*, fASV_24 classified as *Neoascochyta desmazieri* and fASV_118 classified to the genus *Plectosphaerella.* In summary, bacterial and fungal indicators were found for plant community clusters and for fungal community clusters plant and bacterial indicators could be used. In contrast, only fungal and few indicators for bacterial community clusters could be identified.

## 4. Discussion

The assessment of above- and belowground communities in 89 grassland sites in Switzerland revealed that bacterial, fungal and plant communities differed between extensively (unfertilized and late cuttings) and intensively managed (fertilized, early cuttings) grassland. These differences were observed in terms of community structure,

**Table 3**
Number of significant and strong indicators (Indval.g statistic > 0.8) associated to bacterial (B1 and B2), fungal (F1 to F3) and plant (P1 to P3) community clusters and cluster combinations. Taxa for indicator species analyses were selected based on an occurrence in at least 10 sites of a cluster.

| Cluster & cluster combination | Type of indicator taxa | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bacterial ASVs | Bacterial genera | Fungal ASVs | Fungal genera | Fungal primary traits | Fungal secondary traits | Plant species |
| B1 | NA | NA | 48 | 11 | 0 | 0 | 0 |
| B2 | NA | NA | 30 | 3 | 0 | 0 | 0 |
| Total (%) | NA | NA | 78 (3.2) | 14 (3.5) | 0 | 0 | 0 |
| F1 | 639 | 28 | NA | NA | NA | NA | 0 |
| F2 | 11 | 1 | NA | NA | NA | NA | 0 |
| F3 | 578 | 15 | NA | NA | NA | NA | 0 |
| F1 & F2 | 458 | 28 | NA | NA | NA | NA | 1 |
| F2 & F3 | 594 | 47 | NA | NA | NA | NA | 1 |
| F1 & F3 | 3 | 1 | NA | NA | NA | NA | 2 |
| Total (%) | 2283 (16.1) | 120 (13.7) | NA | NA | NA | NA | 4 (10.5) |
| P1 | 54 | 6 | 28 | 12 | 0 | 1 | NA |
| P2 | 0 | 0 | 2 | 1 | 0 | 0 | NA |
| P3 | 11 | 2 | 27 | 3 | 0 | 0 | NA |
| P1&P2 | 8 | 1 | 23 | 19 | 2 | 1 | NA |
| P2&P3 | 87 | 12 | 43 | 5 | 0 | 0 | NA |
| P1&P3 | 20 | 6 | 1 | 4 | 0 | 0 | NA |
| Total (%) | 180 (1.4) | 21 (0.2) | 124 (6.6) | 44 (12.4) | 2 | 2 | NA |

community homogeneity, taxon richness and the degree of similarity between different communities. They were also related to differences in mean plant indicator values for soil nutrients and moisture as well as management related variables. Intensive management and changes in soil nutrient and moisture levels were shown to affect bacterial, fungal and plant communities in grasslands, for example, bacterial and fungal communities in the rhizosphere were more strongly affected by abiotic changes due to land-use intensity than the functional type of the plant from which they were retrieved [44]. Further, a long-term assessment of over 50 years of plant communities revealed a decrease in species richness by 30–50 % mostly due to nutrient input [2]. In a long-term grassland fertilization experiment with different inorganic fertilizers, plant and fungal communities were significantly correlated and both formed distinct communities in treatments including liming or NPK fertilization, while neither of them correlated with bacterial communities, which were affected by the treatments with liming or N fertilization [45].

In addition, there was a significant homogenization effect in intensively managed grassland (more similar communities) in all three organism groups, and this effect was strongest for fungi and plants followed by bacteria. For bacteria, the difference in community dispersion between management intensities was most likely related to a larger soil pH range in extensively (5.2–8) compared to intensively (6–8) managed grassland, because the differences in dispersion were not significant anymore when reducing the dataset to sites with the same pH range for both management types. The sensitivity of bacterial communities to soil pH has been observed in numerous studies [e.g., 10, 28]. Similar to the present study, increased community homogenization with land-use intensification was observed for plant and fungal but not for bacterial communities in grasslands in Germany [6].

Plant and fungal communities also experienced a loss in plant species or fungal ASV richness in intensively managed grassland (Fig. 2), which likely contributed to the homogenization effect, while bacterial ASV richness was even slightly higher in intensively managed grasslands. The decrease in fungal and plant richness may be related to the loss of taxa that are adapted to low nutrients conditions or that are sensitive to frequent mowing and grazing [46].

Management intensity also affected the associations among plant, fungal and bacterial community structures. Correlation strength between pairwise communities was 25–66 % higher in extensively than in intensively managed grassland and remained high even after correcting for pH. The reduction in correlation strength from extensively to intensively managed grassland was more pronounced for the association between plants and bacteria/fungi than between bacteria and fungi. This suggests that intensive management weakens associations and potential interactions among communities, especially between above- and belowground communities. Possibly this is mediated by lower carbon input from plants and subsequent reduced nutrient transfer among soil communities in intensively managed grassland [47] as well as weaker reliance of plants on microbes for nutrients, e.g., nitrogen acquisition [48,49].

The strongest correlations were observed between bacterial and fungal community structures which may be due to their shared habitat of similar scale and/or possibly due to the more similar methodical approach to measure community composition. However, plant communities correlated more strongly with fungal than with bacterial community structures. Also, in a long-term grassland fertilization experiment, plant communities were more strongly correlated with fungal communities while neither plant and bacterial nor fungal and bacterial communities were linked [45]. Yet, on a broader scale, i.e., in a worldwide study on temperate grasslands, communities of plants were significantly correlated with both fungal and bacterial communities after controlling for environmental factors [50]. Compared to the strength of correlations between communities, edaphic, geographic and management-related factors were less strongly correlated with bacterial, fungal and plant communities within extensively and intensively managed grassland, except for soil pH (Table S5). However, even after conditioning for soil pH, strong and significant correlations among community structures were observed, suggesting associations among organisms independent of similar habitat preferences.

Cluster analysis revealed a fungal and a plant cluster (F1 and P2) that had a substantial number of sites in common, i.e., 55.6 % of the sites of P2 were part of F1 and 88.2 % of the sites of F1 were part of P2 and included mostly extensively managed grassland sites, but had a mean grazing intensity, which was closer to the one of the mostly intensively managed grassland clusters (F2 and P3). This suggests that plant and fungal communities reacted differently to grazing intensity alone in comparison to the combined effect of grazing intensity and fertilizer application [46]. In addition, cluster analysis confirmed the strong effect of soil pH and smaller effects of soil nutrients and moisture on bacterial community structures, the impact of management intensity and soil pH on plant communities, and the influence of management intensity, pH and soil nutrients and moisture on fungal communities. The highest number of bacterial and plant indicator taxa were found for fungal clusters, suggesting that both bacterial and plant taxa could serve to a certain extent as proxies for fungal communities. The strongest

indicators for fungal clusters were the bacterial genera *Lacipirellula* (phylum Planctomycetota) and V1-33 (order Verrucomicrobiales), the bacterial ASV bASV_479 (classification to order Rhizobiales) as well as the bacterial genera JAMLHN01 and *Skermanella,* and all of them were associated to a combination of the clusters F2 and F3. Strains from both genera *Lacipirellula* and *Skermanella* have been isolated from terrestrial environments [51,52], but from their taxonomic classification no direct interaction with fungi can be inferred so far from literature. Of the plant species associated to fungal clusters, the highly nutrient-demanding grass species *Poa trivialis* s.l. Was indicative for the combination of F1 and F2, which were the clusters with intensively managed sites and the cluster with extensively managed grasslands but higher grazing intensity. The other quite nutrient demanding grass species, *Poa pratensis*, was associated to the combination of F2 and F3. *Trifolium pratense* s.l. And *Veronica chamaedrys*, which grow in mesic meadows with low or no fertilization, were indicators of the combination of cluster F1 and F3, both with mostly extensively managed grassland sites included. All four species are widely distributed in Switzerland and the plant indicator value of soil nutrient content for both *Poa* species is high (4) while for *T. pratense* and *V. chamaedrys* it is moderate (3) [20]. Potentially these plant species are candidates for proxies (indicators) for fungal clusters. However, whether this is a reliable finding requires further verification.

A higher percentage of fungal than bacterial ASVs were associated to plant clusters and all of the 15 most strongly associated taxa were fungal ASVs, genera and traits, corroborating our finding at the community structure level, in which stronger correlations were found for plant and fungal as compared to plant and bacterial communities. Indicator species analysis revealed that the fungal genera *Hygrocybe* and *Clavaria* were indicative for the plant clusters P2 and P3, of which both contained almost exclusively extensively managed grassland. Six species of the genus *Clavaria* are on the list of protected fungal species and severely endangered in Switzerland [53]. Also, 25 species of the genus *Hygrocybe* are on the red list and 12 of them are classified as vulnerable, 7 severely endangered, 5 potentially endangered and 1 threatened by extinction [53]. This reveals the importance of extensively managed grasslands for the protection of biodiversity. Also, there were indicative fungal taxa that are known for their pathogenicity against plants, for example, fASV_16 classified as *Fusarium graminearum*, fASV_24 classified as *Neoascochyta desmazieri* (formerly *Ascocchyta desmazieri* a pathogen of the grass Lolium spp.) and fASV_118 classified to the genus *Plectosphaerella* [54–56]. They were indicative of the combination of P3 (intensively managed grassland) and P2 (extensively managed grassland with elevated grazing intensity and lower soil pH), suggesting a potential accumulation of pathogens in more intensively managed grassland. However, amplicon sequencing may not sufficiently resolve taxa at the strain level at which differences in pathogenicity are often detected. Therefore, in order to better support the pathogenicity of these taxa, isolates would be required and tested in bioassays. The fungal trait ectomycorrhiza was also among the 15 strongest indicators for plant clusters and was associated to the extensively managed plant clusters, however at rather low relative abundances (below 3 %) suggesting a minor role in the fungal grassland communities. Although, ectomycorrhiza are generally known to be associated to woody plants and more abundant in forests than grasslands, they were found in grassland and negatively correlated to soil nutrients [57]. This may explain their association to extensively managed grassland clusters, which contained lower soil nutrients. Nevertheless, their usefulness as indicators for clusters of extensively managed grassland remains questionable.

## 5. Conclusions

Intensive management, which included higher grazing intensity, higher number of cuts and intensive application of fertilizer had significant effects on bacterial, fungal and plant community structures. This effect was strongest for plant, followed by fungal and bacterial community structures, suggesting plant communities as the most useful management indicators.

A homogenization effect in intensively managed grassland was observed for all three community types and for fungi and plants it was probably related to a loss of taxa. In contrast, for bacteria this was most likely driven mainly by a smaller pH range. Further, a drop in correlation strength from extensively to intensively managed grassland was observed for the correlation of all pairs of organism types suggesting weaker co-occurrence and potentially interactions among them. Furthermore, we detected endangered fungal species that were associated to extensively managed grassland. These results reveal the multifaceted and profound effect of management and underpin the conservation value of extensively managed grassland.

Cluster analysis revealed formerly unnoticed patterns, e.g., a cluster of extensively managed grassland with conditions close to intensively managed grassland, but low pH, and is therefore a helpful tool in biodiversity assessments. Indicator species analyses revealed that certain plant taxa were strongly associated with clusters of fungal communities, revealing their potential of inferring information from plant taxa on fungal communities. Yet, this was not possible for bacterial communities. Conversely, fungal ASVs, genera, primary and secondary traits as well as bacterial ASVs and genera may also serve as indicators for plant community clusters. This means that future monitoring could use fungal components to estimate expected effects on plant communities and vice versa, but bacterial communities would need to be assessed in addition, suggesting that not all components of the biodiversity need to be assessed but it is important to know which types of organisms may follow different patterns.

## CRediT authorship contribution statement

**Johanna Mayerhofer:** Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Franziska Richter:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Aaron Fox:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Franco Widmer:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Andreas Lüscher:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Valentin Klaus:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Martin Hartmann:** Writing – review & editing, Resources, Project administration, Funding acquisition.

## 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

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