Received: 19 November 2021 | Accepted: 10 February 2022

DOI: 10.1111/2041-210X.13865

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Leveraging natural history collections to understand the impacts of global change

Plant biodiversity assessment through soil eDNA reflects temporal and local diversity

María Ariza[1] | Bertrand Fouks[2] | Quentin Mauvisseau[1] | Rune Halvorsen[1] | Inger Greve Alsos[3] | Hugo J. de Boer[1]

[1]Universitetet i Oslo, Naturhistorisk Museum, Oslo, Norway

[2]Westfälische Wilhelms-Universität, Institute for Evolution and Biodiversity, Molecular Evolution and Bioinformatics, Münster, Germany

[3]The Arctic University Museum of Norway, UiT - The Arctic University of Norway, Tromsø, Norway

Correspondence
María Ariza
Email: mariadelosangelesariza@gmail.com

Funding information
H2020 MSCA-ITN-ETN Plant.ID network, Grant/Award Number: No 765000

Handling Editor: Lynsey Rebecca Harper

Abstract

1. Several studies have shown the potential of eDNA-based proxies for plant identification, but little is known about their spatial and temporal resolution. This limits its use for plant biodiversity assessments and monitoring of vegetation responses to environmental changes. Here we calibrate the temporal and spatial plant signals detected with soil eDNA surveys by comparing with a standard visual above-ground vegetation survey.
2. Our approach compares vegetation in an old-growth boreal forest in southern Norway, surveyed in 100 permanent 1-m^2 plots seven times over a 30-year period, with a single soil eDNA metabarcoding-based survey from soil samples collected at the same 100 plots in the year of the last vegetation survey.
3. On average, 60% and 10% of the vascular plants and bryophytes recorded across all vegetation surveys were detected by soil eDNA. Taxa detected by soil eDNA were more representative for the local taxa pool than for the specific plot, and corresponded to those surveyed over the 30-year period although most closely matched the current taxa composition. Soil eDNA detected abundant taxa better than rare ones although both rare taxa and taxa unrecorded by the visual survey were detected.
4. Our study highlights the potential of soil eDNA assessments for monitoring of vegetation responses over broad spatial and temporal scales. The method's ability to detect abundant taxa makes it suitable for assessment of vegetation composition in a specific area and for broad-scale plant diversity assessments.

KEYWORDS
metabarcoding, plant identification, soil eDNA, spatial scale, temporal change, vegetation assessments

1 | INTRODUCTION

The current global warming crisis and the fast pace of global biodiversity losses relative to its appraisal require innovative and rapid operational approaches to biodiversity assessment like never before. Plants are central to most biodiversity assessments, as they are predominant and ubiquitous (Kier et al., 2005), as well as valuable indicators of associated diversity (Brunbjerg et al., 2018), surrounding

abiotic features (Terwayet Bayouli et al., 2021) and human impact (Uuemaa et al., 2013). In addition, plants are suitable organisms for climate changes monitoring as community reshuffling and spatial redistribution towards summits and higher latitudes are well-established biotic responses to increased temperatures (Bertrand et al., 2011; Chen et al., 2011; Steinbauer et al., 2018; Wiens, 2016). Moreover, plant assessments are often required to map habitats, monitor environmental quality and assess habitat changes in space and time (Halvorsen et al., 2020).

Recording the taxonomic composition is the cornerstone of any biodiversity assessment, and this task requires the use of morphological and/or molecular proxies for detection and identification of taxa (Ruppert et al., 2019). Morphological proxies require inspection of plant characters that are diagnostic for the specimens' identity. However, this is a rather lengthy, and thus expensive, process that usually requires participation from trained botanists. In addition, a plant must be noticeably present and/or have the characteristics necessary to enable observation and identification, thus limiting the seasons in which a majority of different taxa present may be monitored. Historically, censuses of plant diversity, forest inventories and monitoring programmes have relied on morphological proxies (Corona et al., 2011). These have contributed greatly to the discovery and current knowledge of the known diversity.

More recently, molecular proxies for taxon identification based on environmental DNA (eDNA) have been taken into use for biodiversity assessment purposes (Beng & Corlett, 2020). The use of eDNA extracted from soil, water, faeces or bulk samples (Taberlet et al., 2012) grants the possibility of collecting organismal or extra-organismal DNA from multiple individuals and taxa simultaneously, saving lengthy and costly hours in the field collecting specimens. In addition, this non-invasive and non-destructive method may be useful for the detection of rare, elusive and/or challenging-to-collect taxa (Alsos et al., 2018; Carrasco-Puga et al., 2021; Hartvig et al., 2021). eDNA-based surveys thus may open for rapid assessment and monitoring of biodiversity within a particular region, which is a critical aspect to understand effects of the current climate change crisis and biodiversity losses. Indeed, soil eDNA samples have gained attention as a potentially valuable tool for the assessment of plant diversity, as it may harbour DNA from both above-ground and below-ground signals (i.e. pollen, debris, roots), from active as well as dormant plant tissues (Hiiesalu et al., 2012). Accordingly, eDNA may provide a series of past and present plant signals that can assist the documentation of local extinctions and long-term ecosystem changes. Soil eDNA has most often been used to assess plant diversity in Arctic and boreal regions where the low temperature facilitates DNA preservation (Edwards et al., 2018; Wang et al., 2021; Willerslev et al., 2014; Yoccoz et al., 2012), although it may also be successful in tropical (Yoccoz et al., 2012; Zinger et al., 2019), and extreme environments such as deserts (Carrasco-Puga et al., 2021) and geothermal sites (Fraser et al., 2018). Furthermore, soil eDNA assessments have been successfully applied to identify present and past diversity from natural or cultivated areas (Yoccoz et al., 2012), assess woody encroachment in grasslands (Sepp et al., 2021) and predict habitats from crime scenes (Fløjgaard et al., 2019).

While eDNA-based detection for assessing diversity is already in widespread use (Deiner et al., 2021), knowledge about the sources, fate, persistence and transport of eDNA in the environment is scarce and mainly explored in aquatic environments (Mauvisseau et al., 2021). Knowledge of these properties of eDNA is indispensable to establish the temporal and spatial resolution expected of an eDNA assessment and to assess the utility of soil eDNA-based methods for ecological monitoring, for example, of biotic responses to climate changes (Deiner et al., 2021). Soil eDNA has been shown to reflect plant diversity at local (Beng & Corlett, 2020; Edwards et al., 2018; Kumpula, 2020; Yoccoz et al., 2012) as well as regional scales (Carrasco-Puga et al., 2021). Furthermore, crop signals from 10 to 50 years into the past have been detected in cultivated soils (Foucher et al., 2020; Yoccoz, 2012). These insights, obtained from diverse environments with different anthropogenic pressures, may indicate that soil eDNA assessments are adequate for monitoring of vegetation. However, exploration of soil eDNA-based methods is still in its infancy and important knowledge gaps still exist as exemplified by the combined effects of spatial and temporal resolution on plant eDNA signals in soils, which to our knowledge has not yet been studied. This and other knowledge gaps have to be filled to establish a calibration field protocol for monitoring vegetation areas in near-natural state, for example, for assessment of vegetation responses to climate changes.

Here, we use time-series data from intermittent vegetation surveys originally designed to track vegetation responses to climate changes in an old-growth forest in South Norway, to investigate the temporal and spatial resolution of a soil eDNA assessment made in 2018. Our research questions are as follows: (a) Do soil eDNA assessments capture the current diversity or integrate diversity over a longer time period?; (b) Do soil eDNA assessments reflect the plot-specific diversity or the local or regional pool of taxa?; (c) Can soil eDNA assessments provide taxa abundance estimates, that is, are common and abundant taxa detected with higher probability than rare, subordinate taxa? We used metabarcoding analysis of the trnL (UUA) intron P6 loop and identified plant sequences with customed reference libraries previously built by sequencing herbarium collections (Alsos et al., 2020; Soininen et al., 2015; Sønstebø et al., 2010; Willerslev et al., 2014). While the marker region is chosen based on vascular plants, bryophytes are common by-catch, and we also report results for bryophytes.

2 | MATERIALS AND METHODS

2.1 | Vegetation plot surveys

In 1988, one hundred 1-m^2 plots were placed across eight transects with a total length of 1,320 m, subjectively selected to cover the broad-scale variation in forest vegetation in response to natural edaphic gradients in the Solhomfjell Forest Reserve, southern

Norway, 58°58′N, 8°58′E, at 350–480 m a.s.l. (Figure 1; Økland & Eilertsen, 1993). Fieldwork permission was granted by the Environmental protection authorities at the County Governor's office in Aust-Agder before fieldwork started in 1988. The studied area is situated within the southern boreal zone and harbours protected old-growth forests with overstories dominated by either Norway spruce (*Picea abies* [L.] H.Karst.) or Scots pine (*Pinus sylvestris* L.), alternating with mires and rock outcrops. The one hundred 1-m^2 plots were distributed semi-systematically along the eight transects. Every 10th m along each transect was a candidate plot position. From the 132 candidate positions, 100 were selected randomly. Of these 100 plots, 61 were dominated by Norway spruce (hereafter referred to as 'spruce subset') and 39 plots were dominated by Scots pine ('pine subset'), respectively. All 1-m^2 plots (hereafter called 'plots') were censused for vegetation composition every fifth year from 1988 to 2018 (t_1 = 1988, t_2 = 1993, t_3 = 1998, t_4 = 2003, t_5 = 2008, t_6 = 2013, t_7 = 2018). At census, all vascular plants (including lignified taxa <80 cm high) and bryophytes were carefully searched for and their presence/absence recorded in each of 16 equal-sized subplots of 625 cm^2 in each plot. Subplot frequency (0–16) was used as a taxon abundance measure. A total of 157 taxa were registered, including 69 vascular plants and 88 bryophytes (Table S1; Figure 1). This time series of vegetation data, hereafter referred to as 'vegetation survey', has been documented and subjected to analyses in a series of publications from the Natural History Museum, University of Oslo (Halvorsen et al., 2019; Økland et al., 2004; Økland & Eilertsen, 1994; Økland & Eilertsen, 1996).

The turnover of the vegetation plot composition was calculated for vascular plants and bryophytes as the sum of proportions of taxa gained and lost from survey time t–1 to survey time t as fraction of the all taxa recorded at the two time points, using the R library 'codyn' (Hallett et al., 2016). Turnover values with 95% confidence intervals for each period were obtained separately for spruce and pine subsets, by averaging across plots.

For all taxa recorded in the vegetation survey, we created a local Solhomfjell sequence reference library for the chloroplast trnL intron (UUA) P6 loop with retrieved sequences from the regional arctic and boreal reference library (Arcborbryo; Soininen et al., 2015; Sønstebø et al., 2010; Willerslev et al., 2014) when available there, otherwise GenBank and for a few taxa unpublished P6 loop sequences were retrieved from PhyloNorway (Alsos et al., 2020, Table S1). Different taxa with identical sequences for this marker were merged at the

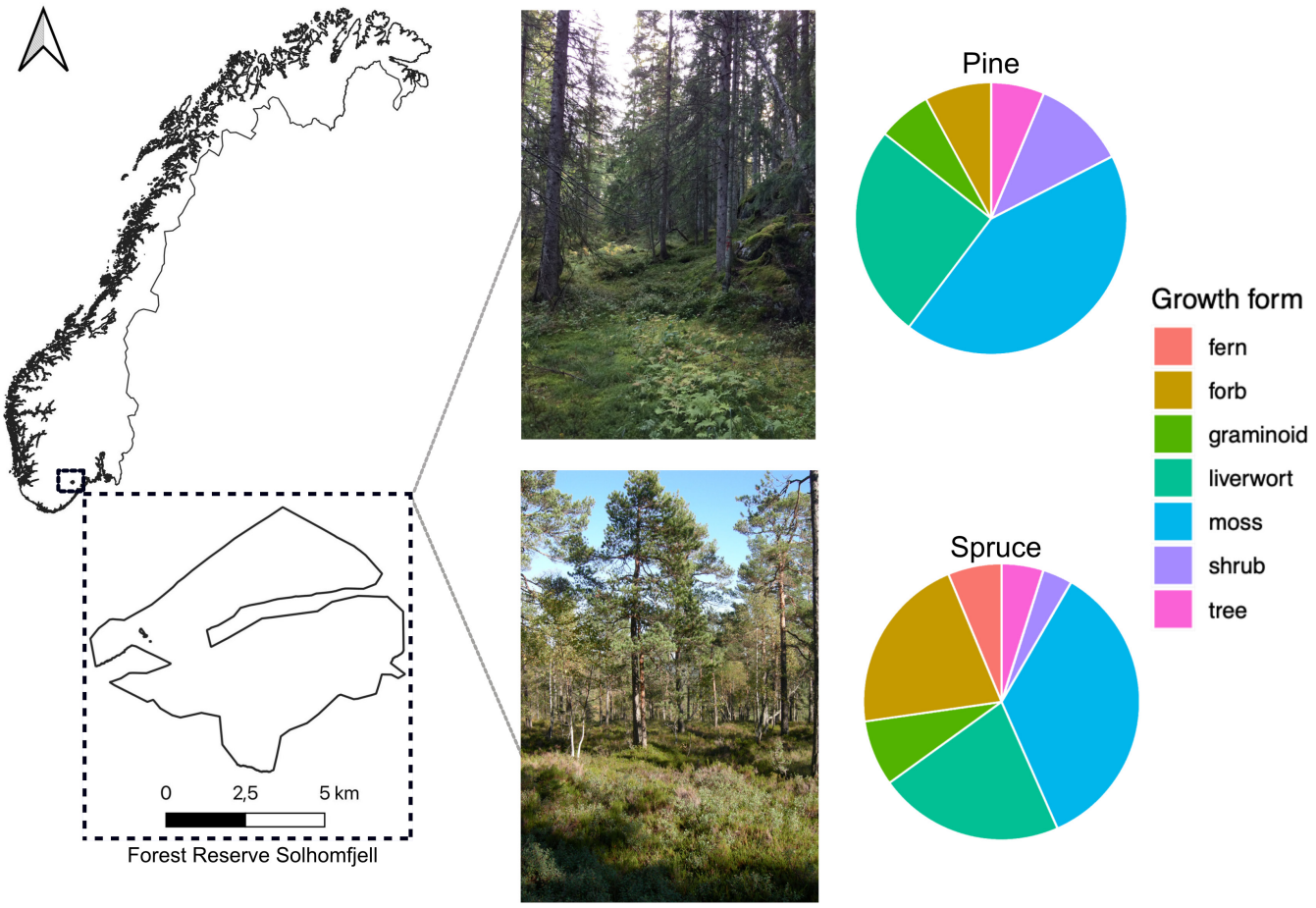

FIGURE 1 Location of the study area, Solhomfjell Forest Reserve, within Norway (left), typical interior of forests dominated by Norway spruce (above) and scots pine (below), and the relative number of taxa of different growth forms registered in the vegetation surveys. A map of the 100 survey plots with specific locations is given in Økland and Eilertsen (1993)

lowest possible taxonomic level and named accordingly. Presence/absence values (1 or 0) for the merged taxon were obtained from the original data by summation of presence values for all parent taxa. A sum ≥1 was scored as presence (1), otherwise absence (0). If each parent taxa had subplot frequency ≥ 16, biomass of the merged taxon was obtained by averaging subplot frequency values for the parent taxa, otherwise the sum of subplot frequency values for parent taxa was used (Table S1).

2.2 | Soil eDNA sampling, amplification and sequencing

A single soil eDNA sample was collected from the centre of each vegetation plot surveyed in the Solhomfjell Forest Reserve in August 2018. Debris and living plant parts were removed to expose the topsoil for sampling, and 50 ml Falcon tubes (11 cm) were pushed into the organic soil. The soil-filled falcon tubes were pulled up and immediately capped after retrieval. The soil eDNA samples were stored in individual plastic bags for transportation to the laboratory and stored at −20 °C prior to freeze-drying under vacuum. Each soil eDNA sample was separately homogenised with ceramic beads and 1 g was used for eDNA extraction. The latter was done in five rounds of two steps: (a) CTAB/chloroform pre-treatment to increase the separation of the organic phase and (b) aqueous phase and using the E.Z.N.A. soil DNA kit following the manufacturer's protocol (Omega Bio-tek; see Data S1 for a detailed protocol). The chloroplast marker trnL (UAA) intron P6 loop was chosen as its short sequence can yield amplification of old DNA material degraded in eDNA samples. This marker was amplified for each sample with the *g* and *h* primers by PCR, using three technical replicates (Taberlet et al., 2007; 5′-GGGCAATCCTGAGCCAA-3′, 5′-CCATTGAGTCTCTGCACCTATC-3′). Forward and reverse primers were tagged with a unique 12 bp oligonucleotide on the 5′ end (Fadrosh et al., 2014). Unique combinations of tagged primers were set up in panels for each PCR reaction for a total of 309 samples (100 samples with 3 PCR replicates each, 5 extractions blanks and 4 PCR negatives). The PCR negatives had no DNA template and were placed on the 96th well position in each panel. Composition of PCRs, final volumes and number of cycles can be found in Data S1. The PCR products were run on a 2% agarose gel, and the amplicon concentrations were measured via band intensity using ImageLab software (Bio-Rad). The lowest concentration (μM) available for all PCR products and its relative volume was identified and the relative concentrations of the PCR products were adjusted to this same concentration. Amplicons were pooled in one library using a Biomek 4000 automated liquid handler (Beckman Coulter Life Sciences). The library was cleaned using AMPure XP reagent beads (Beckman Coulter Life Sciences). The length for all amplicons in the library was determined using a Fragment Analyser (Agilent Technologies). The library was sequenced on an Illumina MiSeq platform with 150 bp paired-end reads (Illumina Inc.).

2.3 | Sequence analysis and taxonomic identification

Sequence data were analysed and curated using OBITools 2 (Boyer et al., 2016; sequences and a detailed script is available at Ariza et al., 2022) following the wolf tutorial with adaptations for demultiplexing dual indexes from QIIME2 (Caporaso et al., 2010). Sequences were retained with both indexes for dereplication for further analysis. Similar sequences were clustered with *obiclean* (Boyer et al., 2016) only when the read count of the less abundant sequence was below 5% of the most abundant sequence. To reduce multiple identifications of the same sequence, taxonomic assignment of dereplicated and denoised sequences was done by matching to three reference sequences databases containing: (a) only taxa registered in the local Solholmfjell reference library (see above); (b) the complete arctic boreal database for vascular plants and bryophytes (Soininen et al., 2015; Sønstebø et al., 2010; Willerslev et al., 2014); and (c) taxa available in the EMBL database (downloaded on 7/02/2020) filtered to sequences with trnL (UUA) intron *g-h* primers using *ecoPCR* tool from OBITools (Boyer et al., 2016). Resulting identifications from the three databases were merged by sequence and duplicates were eliminated giving priority to reference databases (a), (b) and (c) in that order. To minimise erroneous taxonomic assignments, only taxa with a 100% match to a reference sequence were retained. We observed that below this threshold, sequences remained without a taxonomic rank assigned. Furthermore, assigned taxa names were changed to the lowest taxonomic rank possible with trnL (UUA) intron and thus are identical to those registered in vegetation surveys. When different sequences were identified with identical taxa names, a unique entry was retained and the read counts within plots and replicates were summed. Read counts were averaged across all samples and negative controls (extraction + PCR).

2.4 | Comparison between vegetation surveys and eDNA survey

The vegetation survey composition served as a baseline to assess the overlap with the composition of the soil eDNA survey, and from this, the spatial and temporal resolution was derived. Thus, our comparison framework comprises (a) a 7-point time-series of vegetation surveys in one hundred 1-m^2 plots (spruce and pine data subsets) from the Solhomfjell Forest Reserve, carried out in the years 1988, 1993, 1998, 2003, 2008, 2013 and 2018; and (b) a single soil eDNA survey made in 2018 by sampling soil eDNA at the centre of these plots. All the following analyses are plot based, and coded using R v 1.4.17 (R Core Team, 2019) and with packages listed in the code (available at Ariza et al., 2022). Separate analyses are made for vascular plants and bryophytes, and/or for spruce and pine data subsets, or combinations thereof, when relevant.

For comparison between vegetation and soil eDNA survey(s), we quantified the taxonomic overlap for each plot by the *number*

of matching taxa variable, a count of identical taxon names registered in both surveys. Furthermore, for each plot, we calculated the total number of taxa recorded across the seven vegetation surveys by *number of taxa in total vegetation surveys* variable, and the fraction with available reference sequences for the trnL (UUA) p6 loop by *number of detectable taxa in total vegetation surveys* variable. Thus, this variable corresponds to the maximum number of taxa recorded in the vegetation survey that could possibly be identified by soil eDNA. Spearman's nonparametric correlation coefficients (e.g. Sokal & Rohlf, 1995) were calculated between the *number of matching taxa* and *number of taxa in total and detectable vegetation surveys*.

Proportions of *number of matching taxa* out of *number of taxa in total vegetation surveys* and *number of detectable taxa in total vegetation survey* are referred to as *number of matching taxa in total vegetation survey* and *number of matching taxa in total and detectable vegetation survey*, respectively. The proportion of *number of matching taxa in total and detectable vegetation survey* was compared between spruce and pine subsets by use of a two-sample unpaired Wilcoxon–Mann–Whitney rank-sum test (e.g. Sokal & Rohlf, 1995).

2.5 | Temporal resolution of eDNA survey data

The temporal resolution of soil eDNA assessments, that is, at which temporal scale (survey year) the two inventories were most comparable, was evaluated by recalculating per plot the *number of matching taxa* and *number of detectable taxa in vegetation survey t* for taxa compositions from each year of vegetation survey ($t_1 = 1988 \ldots t_7 = 2018$). Then, the proportion of *number of matching taxa* out of the *number of detectable taxa in vegetation survey t* was calculated for each survey, and the seven variables thus obtained per plot are referred to as the *number of matching taxa in detectable vegetation survey t* ($t_1 = 1988 \ldots t_7 = 2018$), etc. An overall assessment was based upon calculation of mean values across all plots with 95% confidence intervals. The 'best detected vegetation survey' (t_{bdvs}) was determined as the vegetation survey *t* with the highest mean plot *number of matching taxa in detectable vegetation survey*. To determine if the mean plot *number of matching taxa in detectable vegetation survey t* was similar across years, multiple pairwise comparisons were tested across all years (groups) using a Friedman Test. A *post-hoc* Tukey test was used to identify significantly different groups.

To determine whether the similarity of the plot *number of matching taxa in detectable vegetation survey t* across years was due to soil eDNA survey recording the same dominant taxa over vegetation survey years, we investigated the relation of this variable to the unchanged composition between survey t_{bdvs} and $t_1, \ldots, t_6$ with a Spearman correlation test. First, we calculated the number of identical taxa between vegetation survey t_{bdvs} and $t_1, \ldots, t_6$ by *unchanged composition* variable. Second, for each plot, we summed the number of taxa between vegetation survey t_{bdvs} and $t_1, \ldots, t_6$ by *taxon count* variable. Thus, six iterations were calculated for both of these variables in each plot. Finally, we calculated the proportion of *unchanged composition* out of the *taxon count* $t_{bdvs} + t$ for each iteration.

To investigate plant DNA permanence and the past plant signals stored in a soil eDNA sample, we annotated each taxon from the plot *number* of *matching taxa in total and detectable vegetation survey* variable with the vegetation survey year *t* in which it was recorded. Since taxa permanence across vegetation survey years will lead to many annotations, we focused on those taxa that were recorded only in 1 year of vegetation survey. We call this subset a *temporal number of matching taxa* and describe how many years ago ($t_1 = 30$, $t_2 = 25 \ldots t_7 = 0$) each taxon was recorded.

2.6 | Spatial resolution of eDNA survey data

The spatial resolution of taxa registered in the soil eDNA survey in a given plot was assessed by categorising each taxon name according to the spatial scale on which was recorded: *matching taxa* in the best detected vegetation survey if recorded within the plot (<1 m^2), *vegetation survey match* if also registered in the best detected vegetation survey but in another plots (>1 m^2), or *regional flora match* if registered in artsdatabanken.no for the 41.77 km^2 Solhomfjell Forest Reserve area (Figure 1; >1 m^2). In addition, taxa with match to non-native plants or with higher mean number of reads in the negative controls than across samples were categorised as *false positives*. Sequence reads assigned to taxa within all categories were counted, and taxon count and proportions were calculated. Moreover, the spatial scales of both soil eDNA and vegetation surveys were compared plot-wise by correlating the number of taxa from above categories to the number of taxa registered in the best detected vegetation survey. Correlations were fitted to a linear model and predicted with a 95% confidence level interval.

2.7 | Representation of vegetation in soil DNA survey

Each taxon name in the best detected vegetation survey was categorised as a *matching taxa* if also present in the soil eDNA survey, *no trnL reference* if the reference sequence for the *trnL* (UUA) intron with *g-h* primers was not available or *undetected by eDNA* if not present in the soil eDNA survey but a reference sequence was available. Taxon counts and proportions within all categories were calculated.

Finally, we investigated if soil eDNA detections of taxa occurring within 1 m^2 plots (*matching taxa*) reflected their abundance. For this, we summarised the subplot frequency (used as proxy for biomass; Porté et al., 2009; Wilson, 2011) separately for *matching taxa* and taxa *undetected by eDNA*. The distribution of biomass data for each taxon in each plot from these two categories was compared with a Spearman's rank correlation test. We also investigated the correlation between the number of reads assigned to each matching taxa within major growth forms and the biomass registered in the best detected survey year.

3 | RESULTS

3.1 | Vegetation surveys

Counts of taxa registered in plots, both in each survey and across all seven vegetation survey years can be found in Data S3. Across all vegetation surveys, the spruce subset registered more unique vascular plants and bryophytes than the pine subset. The number of vascular plant taxa recorded per plot in each vegetation survey was on average 9.23 ± 5.12 *SD* and 5.23 ± 1.40 *SD* in the spruce and pine subsets, respectively, while the corresponding numbers for bryophytes were 9.29 ± 3.33 *SD* and 5.48 ± 2.04 *SD*.

Furthermore, a compositional turnover of 15%–20% and 20%–25% of the vascular and bryophyte composition, respectively, was found between consecutive vegetation survey years (Figure 2). Similarly, higher turnover was found for pine than for spruce subsets for most 5-year periods (Figure 2). Across consecutive vegetation survey years, 7%–15% and 4%–14% of the total bryophytes and vascular plants were gained, and 10%–20% and 10%–15% lost, respectively (Figure S1). Accordingly, the number of taxa of both groups declined over the 30-year survey period. Differences between bryophytes and vascular plants were more pronounced for gains than those for losses (Figure S1).

Reference sequences for the trnL (UUA) intron were available for 84% (133/157) plant taxa from the total vegetation survey. Of the remaining 13% (24/157), 20 were bryophytes and four were vascular plants (Table S1), and these taxa were filtered out from most analyses. Several taxa, 21% (28/133), had identical sequences (mainly bryophytes), and these were lumped into 12 unique taxa. Our final vegetation dataset thus used for comparison with the soil eDNA survey consisted of 65 vascular plants and 68 bryophytes (117 in total; Table S1).

3.2 | Soil eDNA survey

The Illumina MiSeq PE150 run yielded approximately 10 million reads assigned either to plots or blanks (mean reads/plot for all replicates: >100,000; mean reads/blank: 384). Of these, more than 4 million reads matched 100% to 130 taxa in the reference libraries. A summary of the soil eDNA survey is shown in Table S2. The large majority of these reads (4,792,356) were assigned to 116 vascular plants, while only 5,295 reads were assigned to 11 bryophytes. Taxon identifications for vascular plants included 75 species, 31 genera, 8 families and 2 subtribes, while for bryophytes included 6 species and 5 species complexes. Thus, 65% and 55% of the taxa were identified at species level for vascular plants and bryophytes, respectively. The ericaceous genus *Vaccinium*, represented by *V. myrtillus*, *V. uliginosum* and *V. vitis-idaea*, conifers (*Pinus sylvestris* and *Picea abies*), and deciduous trees (*Betula* spp, *Populus tremula*) were the most common vascular plants found in the eDNA records. The most common bryophytes found in eDNA records were the feather mosses *Hylocomium splendens* and

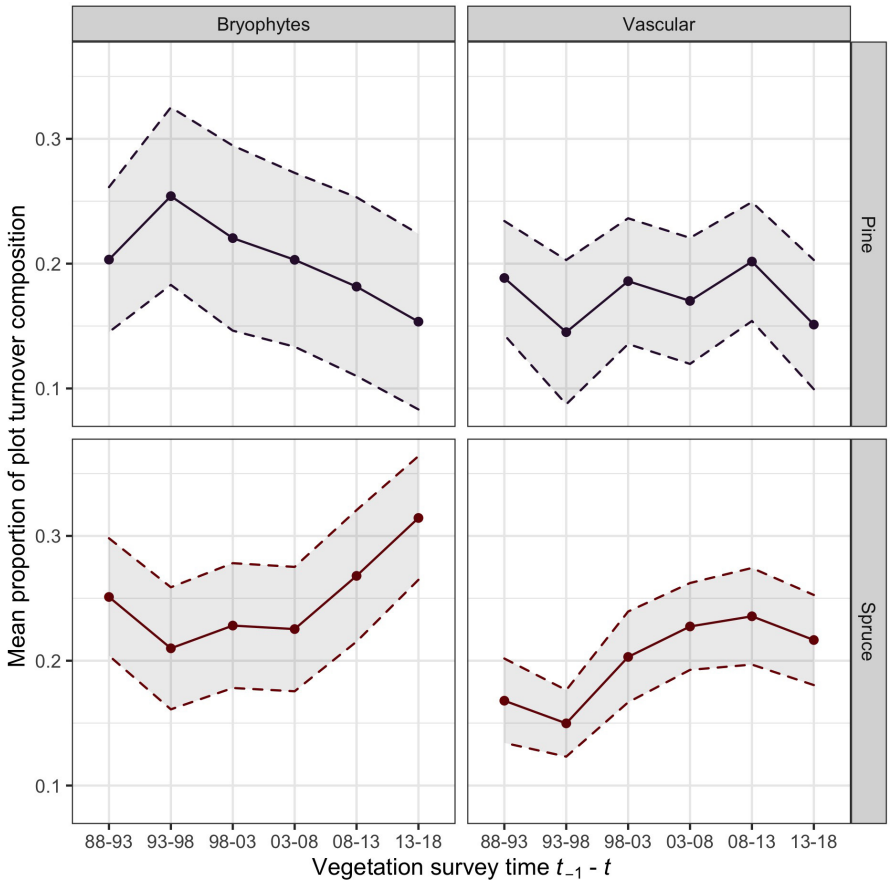

FIGURE 2 Compositional turnover over 5-year periods based upon the vegetation surveys, expressed as averages of plot values for total turnover and calculated separately for pine and spruce subsets. Confidence intervals (95%) are indicated by grey shaded areas delimited by dotted lines in the respective colours

Pleurozium schreberi. The mean number of vascular plants registered in the soil eDNA survey per plot differed among subsets but was similar for bryophytes (spruce forest subset: vascular plants: 37.46 ± 11.36 *SD*; bryophytes: 3.27 ± 2.21 *SD*; pine forest subset: vascular plants: 29.66 ± 9.08 *SD*; bryophytes: 4.41 ± 2.51 *SD*; Table S3).

3.3 | Matching taxa and temporal resolution of eDNA survey data

A total of 53 vascular plants and 11 bryophytes were registered both in the vegetation and the eDNA survey (*matching taxa*), accounting for 81% (53/65) and 13% (8/68) of the total vegetation survey, respectively (a summary is found in Table S3). The mean proportion values from the *number of matching taxa in the total and detectable vegetation survey* per plot were 0.60 ± 0.18 *SD* for the vascular and 0.10 ± 0.12 *SD* for bryophytes, respectively. For both taxa groups, the *number of matching taxa* and the *number of detectable taxa in total vegetation survey* were positively correlated (vascular plants: Spearman's $\rho = 0.891$, $p = < 2.2e\text{-}16$, $n = 100$; bryophytes: Spearman's $\rho = 0.219$, $p = 0.028$, $n = 39$; Figure 3). The proportion of *number of matching taxa in total & detectable vegetation survey* did not differ between spruce and pine subsets (Wilcoxon–Mann–Whitney rank-sum tests: pine subset: $W = 1{,}752$, $p = 0.632$; spruce subset: $W = 1{,}963$, $p = 0.274$; Figure S2).

Plot-wise, the *number of matching taxa* calculated for the soil eDNA survey with respect to each of the seven detectable vegetation surveys (t_1, ...,t_7) varied between 0.55 and 0.7 across years for vascular plants, and between 0.10 and 0.20 for bryophytes (Figure 4). For both taxonomic groups, the highest mean *number of matching taxa in detectable vegetation survey t* was observed for the last vegetation survey ($t_7 = 2018$), the year soil eDNA was sampled (Figure 4). Thus, subsequent analyses were made only comprising taxa from plot compositions registered on the 2018 vegetation survey and referred to as the 'best detected vegetation survey' ($t_{bdvs} = t_7 = 2018$). However, the mean *number of matching taxa in detectable vegetation survey t* for vascular plants only differed significantly between four last vegetation surveys and the first survey made (Figure 4; Friedman test: $\chi^2 = 24.005$, $df = 6$, $p = 0.0005211$; *Post-hoc* Tukey test: t_7–t_1: z = 4.137, $p < 0.001$, t_6–t_1: z = 3.577, $p = 0.006$, t_5–t_1: z = 3.074, $p = 0.034$; t_4 = –t_1: z = 3.076, $p = 0.341$) and, for bryophytes, significant differences were found only between the last and the three first surveys (Figure 4; Friedman test: $\chi^2 = 41.35$, $df = 6$, $p < 0.001$; *Post-hoc* Tukey test: t_7–t_1: z = 3.409, $p = 0.0117$, t_7–t_2: z = 4.499, $p < 0.001$, t_7–t_3: z = 3.790, $p = 0.003$). The low *number of matching taxa in total and detectable vegetation survey* resulted from 132 and 142 unique appearances of vascular plants and bryophytes across the seven individual surveys, respectively (Figure 4).

Moreover, in each plot, the proportions of unchanged composition between survey t_{bdvs} and t_1, ...,t_6 varied from 0.4 to 1 for vascular plants. However, these proportions were not correlated to the

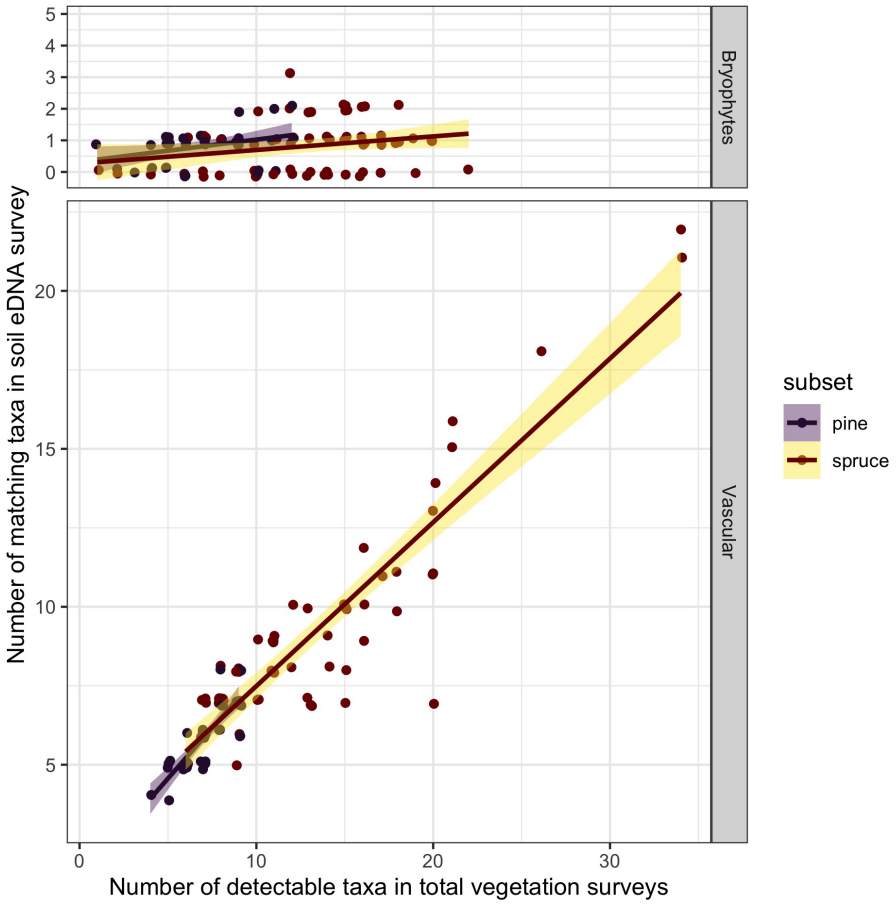

FIGURE 3 Matching taxa between the total vegetation survey and the soil eDNA survey. The total number of detectable taxa in each 1-m^2 plot of the spruce or pine subset registered across seven vegetation surveys is related to the number of identical taxon names recorded in the soil eDNA survey. Lines represent linear models for *number of matching taxa in soil eDNA survey* regressed on *the number of detectable taxa in total vegetation survey*; shaded areas are 95% confidence level intervals for model predictions. To avoid spatial overlap points are jittered by up to 0.15 units along both axes when necessary

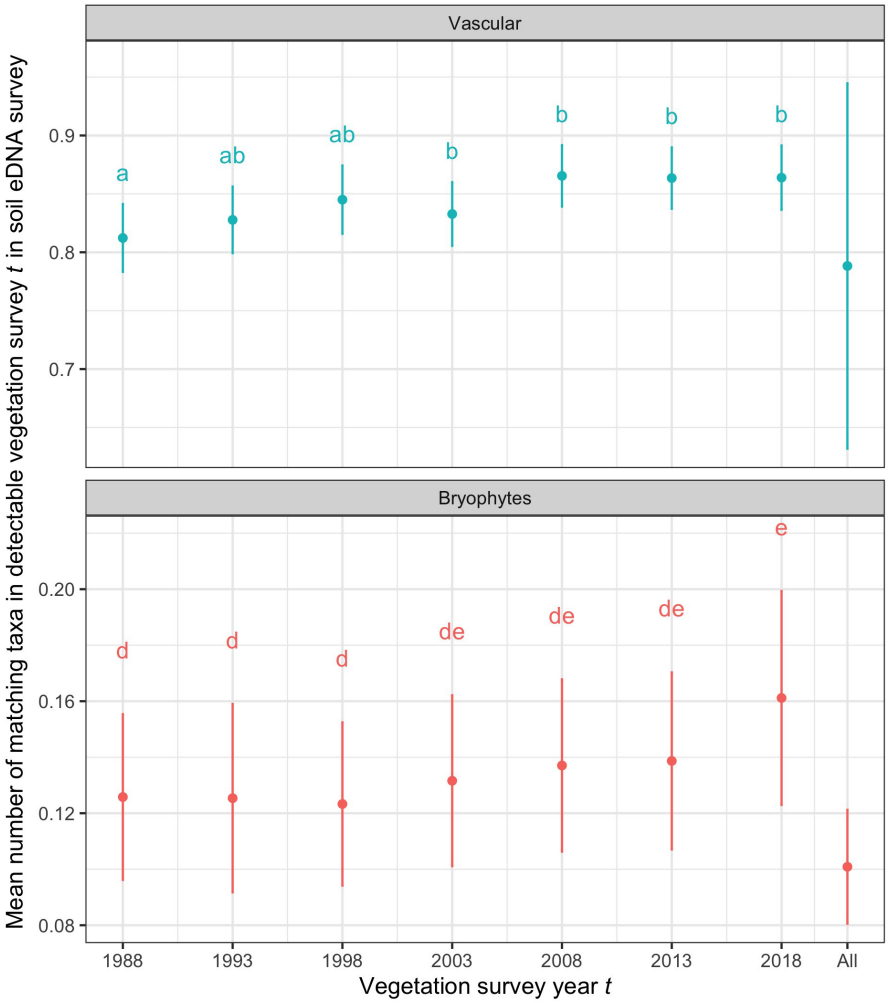

FIGURE 4 Matching taxa in each detectable vegetation survey and temporal resolution of soil eDNA survey. The number of matching taxa (the number of identical taxon names registered in both detectable vegetation and soil eDNA surveys) are calculated per plot for each plant group, and for compositions from each vegetation survey (t_1, ...,t_6) and for the total vegetation survey ('all'). The proportion of matching taxa in a vegetation survey *t* is the fraction of number of matching taxa out of the detectable taxon count in a vegetation survey t or in the total vegetation survey. Points and bars indicate means with 95% confidence intervals for the means. Identical small letters indicate non-significant differences ($p > 0.05$) in pairwise Friedman multiple comparison tests

proportion of matching taxa calculated for the same plot (Figures S3; Spearman's $\rho = -0.041$, $p = 0.316$). Conversely, for bryophytes, the plot proportions of unchanged composition between survey t_{bdvs} and t_1, ...,t_6 varied from 0 to 1 and these were slightly correlated to the proportion of matching taxa (Figures S3; Spearman's $\rho = 0.216$, $p < 0.05$).

Furthermore, the mean number of vascular plants and bryophytes that were registered only in a vegetation survey year were 18.57 ± 14.70 *SD* and 20.28 ± 7.27 *SD*, respectively (a summary for each vegetation survey *t* is found in Table S4). Consequently, the subset *temporal number of matching taxa* contained 40% of vascular plants from each vegetation survey year *t* and 6% of bryophytes only for years 1988, 1998 and 2003. Trees such as pine *Pinus sylvestris*, birch (*Betula* spp.) and European aspen *Populus tremula* were most common across years in this *temporal number of matching taxa* subset, but taxa from all plant forms were also detected.

3.4 | Spatial resolution of eDNA survey data

When comparing the soil eDNA survey to the best detected vegetation survey ($t_7 = 2018$), the majority of records of both vascular plants and bryophytes matched the vegetation survey at the 1-m^2 plot scale (*matching taxa*) or, at broader scale, other taxa from the total vegetation survey (*vegetation survey match*; Figure 5a). The large majority of sequence reads were assigned to taxa from these two categories (Figures S4). In addition, on average, 5.94 ± 2.70 *SD* vascular plants per plot from soil eDNA survey were not registered in any vegetation survey but were known from the Solhomfjell Forest Reserve (*regional flora match*; Table S3). The total number of taxa in this group was 51. These are mainly perennial shrubs, herbs and graminoids typical of boreal forests (Table S3 for a taxonomic overview and descriptive statistics). False positives, all vascular plants, were registered in 61 plots accounting for 8% of the soil eDNA survey (10/129) and less than 3% of the total reads (Figure S4).

For vascular plants, number of taxa registered in the best detected vegetation survey ($t_7 = 2018$) was positively correlated with *matching taxa* (Spearman's $\rho = 0.762$, $p < 0.001$, $n = 100$) and *regional flora match* variables (Spearman's $\rho = 0.375$, $p < 0.001$, $n = 100$), while the *vegetation survey match* (Spearman's $\rho = 0.088$, $p = 0.381$, $n = 100$) and false positives (Spearman's $\rho = 0.154$, $p = 0.125$, $n = 100$) were not significantly correlated. For bryophytes, positive correlations were found for *matching taxa* (Spearman's $\rho = 0.157$, $p = 0.117$, $n = 100$), whereas a negative correlation was found with *vegetation survey match* (Spearman's $\rho = 0.088$, $p = 0.380$, $n = 100$). A summary figure is found in Figure S5.

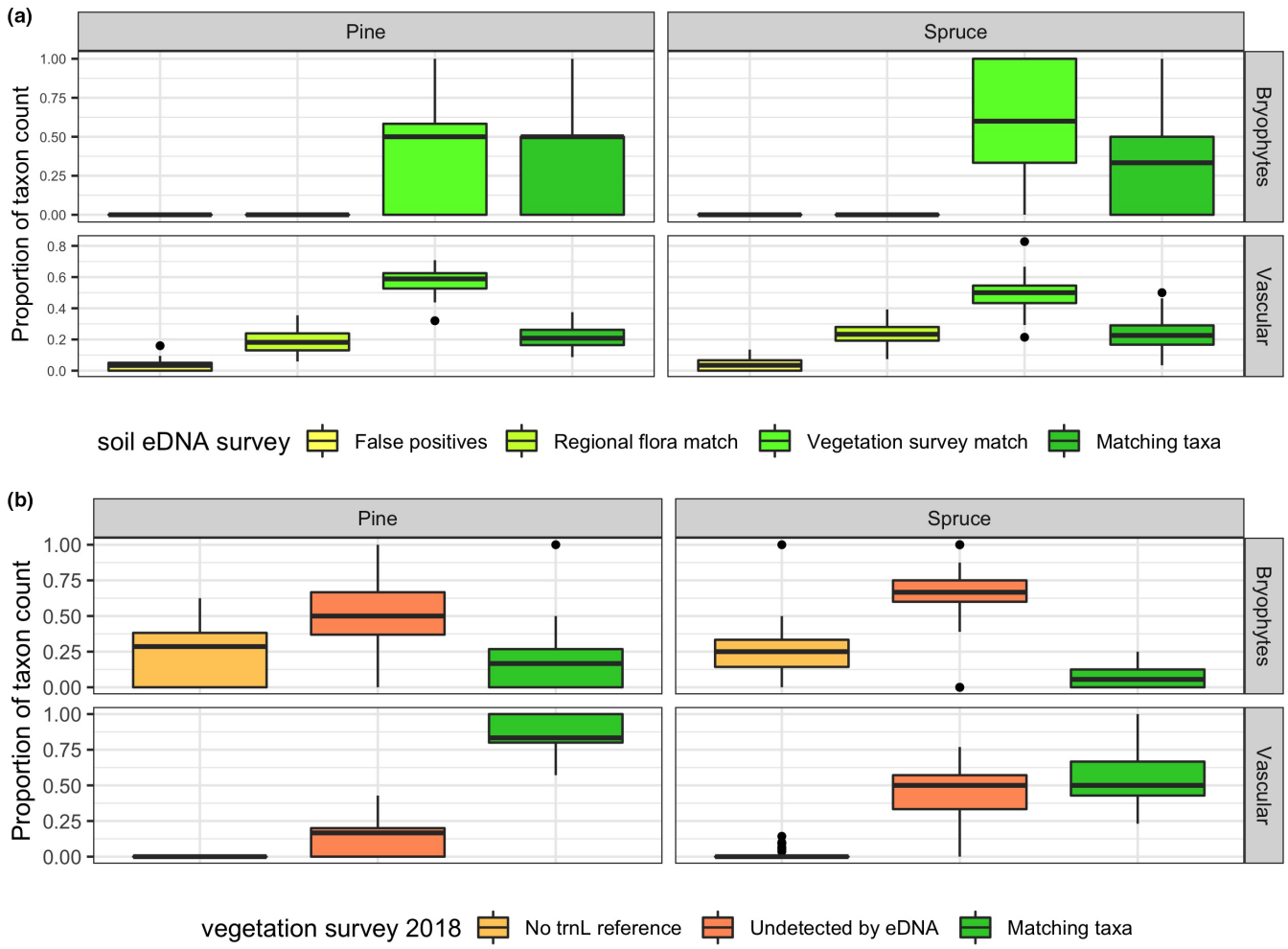

FIGURE 5 Spatial resolution of the soil eDNA survey and best detected vegetation survey. (a) Plant taxa detected with a single soil eDNA sample from each plot is assessed according to the spatial scale on which was recorded by categorising into: Matching taxa (taxa registered in the plot in the best detected vegetation survey $t_7 = 2018$); vegetation survey match (taxa recorded in any other plot in the best detected vegetation survey $t_7 = 2018$); regional flora match (taxa recorded in the Solhomfjell area outside plots); and false positives (taxa not recorded in the Solhomfjell area or with higher mean read count in PCR negatives than across samples and replicates). (b) Plant taxa recorded in each plot at the last vegetation survey $t_7 = 2018$ are categorised into: Matching taxa (taxa also registered in the soil eDNA survey); undetected by eDNA (trnL reference sequence available, but taxa were not registered in the soil eDNA survey); and no trnL reference available (no reference sequence was available for identification with eDNA data). For both panels, results are shown for taxon count proportions from each category and subsets with boxplots: horizontal bold lines depict median, the boxes show interquartile range and vertical lines indicate non-outlier range; the dots represent the outliers

3.5 | Representation of vegetation in soil DNA

On average, 6% ± 21 *SD* and 16% ± 19.00 *SD* of the vascular plants and bryophytes proportion registered in the best detected vegetation survey ($t_7 = 2018$), respectively, were also present in the soil eDNA survey (*matching taxa*; Figure 5b). The full vascular plant composition of this vegetation survey was recovered by the soil eDNA survey for 13 and 3 plots in the pine and spruce forest subsets, respectively (Figures S6). The majority of bryophytes recorded in the vegetation survey belonged to the *undetected by eDNA* category while for vascular plants the proportion of this category accounted for 0.32 of all taxa (Figure 5b). The proportion of vascular plants with *no trnL reference available* accounted for less than 0.03 of the total composition from only in six plots of the spruce subset, whereas for bryophytes this category accounted for more than 0.30 across plots of both pine and spruce subsets (Figure 5b).

The abundance of taxa registered in the best detected vegetation survey ($t_7 = 2018$) was significantly higher for taxa detected with soil eDNA than for taxa undetected by soil eDNA, for both vascular plants and bryophytes (Wilcoxon–Mann–Whitney rank-sum tests: vascular plants: $W = 19{,}386$, $p < 0.001$; bryophytes: $W = 16{,}986$, $p = 0.0087$; Figure 6). Sequence read counts for each taxa of ferns, forbs, mosses and trees taxa were slightly positively correlated to the respective abundances registered in the best detected vegetation survey, while more strongly positive correlations were found for graminoids, herbs and shrubs taxa (summary figure is found in Figure S7).

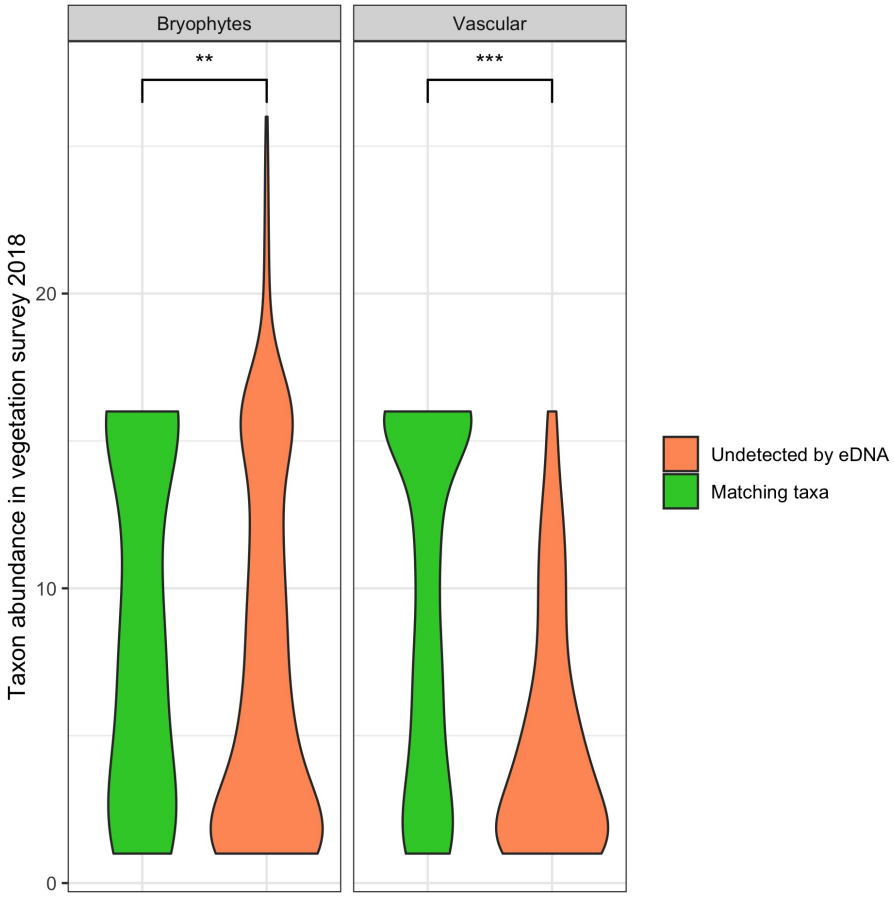

FIGURE 6 Correspondence between taxon detectability and abundance in the year of the best detected vegetation survey (t_7 = 2018). The composition of each plot from the best detected vegetation survey is categorised as matching taxa and undetected by eDNA. The distribution of summed subplot frequency (proxy for biomass) from each taxon in these two categories is shown by violin plots: Shape width is proportional with the frequency of observations. Subplot frequency corresponds to taxon presence in 625 cm^2 grids, and values >16 correspond to taxa with identical trnL (UUA) intron sequences that were merged into one single taxon with their subplot frequencies summed. Significance of differences between biomass from taxa within both categories was tested using a Wilcoxon–Mann–Whitney rank-sum test and are shown with ** and *** for p values <0.05 and <0.01, respectively

4 | DISCUSSION

4.1 | Vegetation survey inventories and its change across time

More bryophytes than vascular plants were registered in plots from the spruce subset than pine subset (Figure 1), and its average taxon counts in the total vegetation survey follow the same plot trends across all survey years (Data S3). Overstory dominance by pine or spruce influences the understory vegetation by different effects on ground-level light conditions (Felton et al., 2020), with lower light levels in the spruce forest (Esseen et al., 1997). Reduced richness in pine forest from the Solhomfjell area has been attributed to severe effects of drought from its shallow soils (Økland & Eilertsen, 1996). Furthermore, nutrient-demanding taxa are restricted to the richer and deeper soils that in the Solhomfjell area are found in spruce forests only (Økland & Eilertsen, 1993). Dwarf shrubs such as bilberry and lingonberry, and mosses such as feather moss and shaded wood-moss, registered in the majority of plots, are common and dominant species in boreal understory forest vegetation (Nilsson & Wardle, 2005; Økland et al., 2004).

An average of one to two vascular plants and bryophytes were either lost or gained between consecutive vegetation surveys, respectively, and fluctuations observed in plots located below overstories dominated by pine were generally lower than spruce (Figure 2). Both trends were also observed in Solhomfjell plots analysed every year from 1988 to 1993 (Økland & Eilertsen, 1996), and in other boreal forests in the south of Norway between 1988 and 2003 (Økland et al., 2004). This suggests these turnover rates are representative for Norwegian boreal forests over the last 30 years. The latter studies also detected a decrease in vascular plant richness, notably in spruce forests, and increase in large bryophytes in both forest types, attributed to a combination of past soil acidification due to higher deposition of air pollutants and longer and warmer growth seasons. Though we did not detect steady trends of gain or loss of vascular plants and bryophytes between consecutive years of vegetation survey, we detected peaks that may correspond to exacerbation of these climatic conditions (Figures S1).

4.2 | Soil eDNA survey and detection of taxa

The soil eDNA survey consisted of 127 taxa assigned mostly to species level (63.77%), from which 53 were vascular plants not registered in the total vegetation survey (Table S2). In all, 57 taxon detections accounted for 81% and 13% of vascular and bryophyte taxa registered in the total vegetation survey, respectively (Table S2). Soil eDNA surveys in temperate and tropical forests, tundra and deserts have routinely found 'hidden taxa' that were not observed when surveying above-ground diversity using vegetation surveys (Carrasco-Puga et al., 2021; Edwards et al., 2018; Osathanunkul et al., 2021; Palacios et al., 2021; Yoccoz et al., 2012). Small herbaceous plants

and seedlings can be easily overlooked while surveying vegetation, and eDNA-based assessments detect DNA rather than organisms (Deiner et al., 2017). The pool of plant DNA in top soils is composed of locally deposited debris, roots, rhizomes and seeds. In addition, it may contain local and/or exotic pollen, but this does not seem to contribute to the local eDNA signal (Edwards et al., 2018). Metabarcoding analysis of root diversity has highlighted the DNA contribution of many perennial plants that persist below-ground even in the temporary absence of above-ground parts, which, in turn, increases below-ground richness estimates compared to above-ground (Pärtel et al., 2012; Rucińska et al., 2022; Träger et al., 2019). In our study, soil eDNA detections of taxa not registered in the vegetation surveys but present in the Solhomfjell area are indeed mostly perennial plants (Figure 5a; Table S2), suggesting that these are most likely local signals from plants growing in between vegetation survey plots or seedlings not recruited in the plot.

Reference sequences for the trnL (UUA) intron P6 loop were available for about 95% and 75% of the vascular and bryophyte taxa registered across all vegetation surveys, respectively, and the taxonomic resolution was 75% and 65% identified to species level. While longer markers such as ITS, matK and rbcL in general may provide higher taxonomic resolution, the actual taxonomic resolution obtained depends on the marker region used, the representation in the reference library and the size and nature of the local flora (Hollingsworth et al., 2016). The P6 loop of the chloroplast trnL (UUA) intron (Taberlet et al., 2007) is the most commonly used marker for soil eDNA studies targeting vascular plants (Capo et al., 2021; Parducci et al., 2017), as its short sequence may be found in the degraded DNA that is typically present in underground decomposed material and sediments (Taberlet et al., 2007). This primer is designed to target vascular plants, and our results on vascular plants show high detection and high taxonomic resolution similar to other studies that are based on this primer (e.g. Alsos et al., 2018; Edwards et al., 2018). Other primer pairs for the P6 loop such as *c-d* (Taberlet et al., 2007) and Bryo_P6 (Epp et al., 2012) are conserved from Bryophytes to Angiosperms, but these markers have not been widely used and very few reference sequences exist in public repositories (Boukhdoud et al., 2021; Soininen et al., 2017). Though other nuclear ribosomal (ITS) and chloroplast (rbcL) markers may yield higher specificity in bryophytes (Lang et al., 2014; Liu et al., 2010), detection of bryophytes with eDNA-based assessments is limited by low intraspecific variation in marker regions (Hassel et al., 2013) and particularly for boreal forests when relying on non-exhaustive reference libraries. However, the fact that we did detect bryophytes in almost every soil eDNA sample suggests that there is a great potential for eDNA also for this group, but we recommend further development of primer design and build up of reference library

The detection of taxa was related to abundance, which, in turn, is an expression of biomass. The soil eDNA survey failed to detect some taxa registered in vegetation surveys even when reference sequences were available (Figure 6), but most of these had significantly lower plot abundance than those that were detected (Figure 6). A positive relation between detectability and plant biomass has been also observed in tundra and temperate sites (Alsos et al., 2018; Edwards et al., 2018; Yoccoz et al., 2012) and in aquatic environments (Alsos et al., 2018; Anglès d'Auriac et al., 2019; Matsuhashi et al., 2016). The relation is often attributed to the greater chance of deposited or suspended plant DNA that can be detected with higher organismal biomass. However, our study also reports detections of taxa present in only one out of the 16,625 cm^2 subplots of a vegetation plot (Figure 6), demonstrating that soil eDNA metabarcoding also detects some rare taxa. In addition, the apparent stochasticity of rare taxa raises questions on how biomass differences between root and shoot at different life-history stages (Qi et al., 2019) can potentially underlie the detection of less abundant taxa. Furthermore, in metabarcoding studies, sequence read counts are often interpreted as a proxy for abundance since DNA template availability for PCR amplification covariates with biomass (Amend et al., 2010; Beng & Corlett, 2020; Deagle et al., 2019). However, the signal of biomass can be diluted by technical and biological biases in marker recovery rates across different taxa (Deiner et al., 2017). In our study, sequence read counts assigned to taxa from all considered growth forms registered in the last year of vegetation survey correlated positively to their plot abundance, and these correlations were significant for forb, graminoid, moss and shrub taxa as well as for all life-forms combined (See Figure S7 for figures and *p* values). The correlations for ferns and trees were not significant, and this might be due to the abundance being skewed by the larger aerial vegetation cover in relation to the smaller underground cover of these taxa, as may decrease the DNA contribution of roots and rhizomes to the soil eDNA pool in relation to other growth forms (Qi et al., 2019).

4.3 | Matching taxa and temporal calibration

Each plot has a measure of the total number of unique taxa registered during the seven survey years as well as the number of taxa detected in the single eDNA survey. On average, 60% and 10% of the vascular and bryophyte taxa from the total vegetation survey matched with a single soil eDNA survey, respectively (Figure 4; Table S3). Conversely, 18% and 30% of the vascular and bryophyte taxa registered by a single soil eDNA survey matched with the total vegetation survey, respectively (Table S3). Similar rates of undersampled vegetation, that is, taxa that were detected in only one of the two surveys (Edwards et al., 2018), and matching taxa values, have been found when comparing surveys at similar and even larger plot scales (1-m^2 plots in alpine subarctic vegetation Kumpula, 2020; 1–4 m radius from circular plots in Svalbard tundra Edwards et al., 2018; and 15-m^2 plots in Varanger boreal forest Yoccoz et al., 2012), highlighting the ability of both proxies to assess the total vegetation of a site. In our study, we show that the number of matching taxa increased with richness registered in the total vegetation survey in both pine and spruce subsets (Figure 3), and this suggests that differences in soil and vegetation properties in these two environments probably have no effect on detectability. Furthermore, surveys built by both

proxies detected the same dominant taxa. This highlights the ability of both proxies to recover the main components of the vegetation.

Our soil eDNA survey made in 2018 on average matched best with the plot composition surveyed the same year. This holds true for vascular plants as well as for bryophytes. Roots and shoots from live plants and derived litter are probably the biggest contributors to plant DNA in the soil, and also the least degraded and therefore more likely to be detected. Although the match between vascular plant compositions registered each vegetation survey year and the soil eDNA survey were similar, these similarities were not correlated to soil eDNA detections of composition persisting (or unchanged) across surveys (Figure S3; Figure 2). Soil eDNA detections of vascular plants registered uniquely at a vegetation survey *t* also support this (Table S4). Altogether, our study indicates that a single soil eDNA survey can detect taxa from multiple vegetation surveys and its turnover fraction across time with the same power, and pinpoint how soil eDNA samples can encapsulate the vascular composition going back at least 30 years. Detections of past signals from 30 up to 50 years ago have also been found in crop soils (Foucher et al., 2020; Yoccoz et al., 2012), though these are more likely to be detected since plant biomass exponentially increases in monocultures. However, unique past signals detected in our study correspond to taxa with median to low abundance, registered in 50% or fewer subplots. This suggests that biomass may not play a role in the detectability of past signals in natural environments (Data S4). Nevertheless, detections of past signals can be also attributed to the resurfacing of deep soil particles through bioturbation by biotic underground DNA transporters such as insects, moles, worms, etc. (Prosser & Hedgpeth, 2018). The match with the composition of bryophytes registered in the best detected vegetation survey ($t_7 = 2018$) was significantly higher than with the rest of the vegetation surveys and significantly correlated with the proportion of unchanged taxa (Figures S3). This indicates that the soil eDNA survey mainly detected a similar fraction of bryophyte composition from each vegetation survey. Mosses and liverworts are poorly detected, and this is probably due to a combination of factors including mismatch of the trnL (UUA) intron *g-h* binding site for these taxa, but also that their DNA is probably underrepresented in the soil pool in comparison to that of vascular plants as most of their biomass is allocated in the forest floor making them less detectable over years (Bergamini et al., 2001).

4.4 | Spatial patterns of detection

On average, about 55% of the soil eDNA survey composition in each plot matched the composition registered in any other (near) plots or in the Solhomfjell area, whereas about 22% matched the vegetation plot composition (Figure 5a), suggesting that soil eDNA samples reflect mainly local vegetation rather than plot specific signals. Our results contrast those from Edwards et al. (2018) in Svalbard tundra where soil eDNA signals were highly specific to those recorded within a circular plot of 1 m radius and no taxa existing beyond a 4 m radius were found. Instead, our results are more in concordance with <1 km signals speculated by Yoccoz et al. (2012), as these authors did not find signals in uncultivated meadows from crops located a kilometre away but found signals that are likely part of the regional species pool. Differences in taxa richness and vegetation distribution between tundra (low, homogeneous) and temperate forests (high, heterogeneous) such as that of Solhomfjell area led us to consider how distribution patterns of vegetation may hinder the spatial recovery of soil eDNA signals. Furthermore, the hilly Solhomfjell landscape in which the plots are located may contribute to DNA transport from one plot to another via snow-melt, rainfall run-off and through-flow, thus enabling detections from other plots. Although we attempted to calibrate each eDNA detection with a match to an area, that is, a match to the vegetation survey is a match to an area >1 m^2, our categories may disguise a temporal match within plots (a match to taxa detected in the same plot back in time). Our study is the first to assess simultaneously the temporal and spatial resolution of soil eDNA samples in natural environments, yet our approach cannot disentangle the contribution of each signal separately. This limitation highlights the need for studies in controlled microcosms where plant signals can be followed with biomarkers, thus enabling the possibility of tracing both the spatial and temporal signals. Nevertheless, our taxa comparison in space allowed us to identify plant richness detected by both methods with similar sampling efforts (surveying 1-m^2 plot vs taking a soil sample at the centre of 1-m^2 plot; Figure S5) and so provide a baseline for decision-making when designing sampling for soil eDNA assessments.

4.5 | Limitations and considerations for soil eDNA for plant diversity assessments

Soil and sediments are suitable substrates for eDNA-based plant assessments in terrestrial environments, as most extra-organismal and organismal plant DNA from both active and dormant tissues are gathered or ultimately deposited in these substrates (Rodriguez-Ezpeleta et al., 2021). Accordingly, soil eDNA assessment is a valuable tool for identification of plant diversity at any season, especially when non-destructive and easy sampling is needed. Our study has shown how a single eDNA sample can signal local dominant flora and thus might be useful for general plant diversity assessments. However, when detection of less abundant and/or rare taxa is desired, collecting multiple eDNA soil samples is recommended. Moreover, our study shows how a single soil eDNA survey can provide a series of local, regional, past and present plant signals that can help track long-term responses to climate and ecosystem changes. However, as with any method, there are some limitations to consider before embarking on a plant soil eDNA study (see Figures S8 for a summary of methodological steps). Since eDNA-based organismal detections are dependent on both DNA presence (intracellular or extracellular) and environmental conditions that may enhance or diminish DNA permanence, degradation and/or decay (Nagler et al., 2018; Pietramellara et al., 2009; Rodriguez-Ezpeleta et al., 2021), an evaluation of the potential state of DNA given the study environment is

essential before choosing an appropriate approach. Plant eDNA from soil substrates is particularly subject to degradation or decay from decomposition processes of organic matter by both underground and above-ground biota (Pietramellara et al., 2009). Thus, long DNA fragments are expected to account for the lowest fraction of target soil eDNA that can be isolated. In tropical areas, warmer environments and richer decomposing communities may exacerbate DNA degradation rates in soils (Pietramellara et al., 2009). Furthermore, DNA decay increases with time and past plant signals may be only in the form of short DNA fragments (Kistler et al., 2017). For these reasons, it is generally recommended to employ markers targeting short DNA sequences. Indeed, most plant eDNA-based studies employ the chloroplast trnL (UAA) intron which amplifies on average a 50 bp region that has been robustly catalogued for the flora of Fennoscandia (Alsos et al., 2020). Noteworthy, in temperate areas, studies have successfully amplified matK, rbcL, ITS2 markers with target regions of more than 490 bp (Fahner et al., 2016). Furthermore, the use of short markers may be hindered by their low variability across species resolutions, that is, that identification of related taxa is supported by a few base pairs only (Taberlet et al., 2007). Employing short markers may thus require stricter thresholds of OTU (head sequence) matching to a reference sequence as the probability of identification mismatching resulting from polymerase errors is amplified. Although the prospecting of new plant DNA markers with targeted capture of multiple informative genes is promising, eDNA-based assessments can only identify taxa present in a reference sequence library. Thus, an eDNA assessment is only as good as its reference library. Although correlations between plant biomass and DNA concentration in the environmental samples are poorly understood, several studies—including this one—show that read counts may be used as a proxy for biomass of some plant life-forms (Deagle et al., 2013; Deiner et al., 2021). Finally, as DNA may remain in the environment after the organism is no longer present (Harrison et al., 2019), taxon detections provided by eDNA-based assessments should be interpreted merely as detections of organismal DNA. If one is interested in detections of live organisms, RNA approaches should be considered. These limitations highlight the importance of considering the current literature carefully to ensure that the study design is suited to address feasible and measurable questions.

5 | CONCLUSIONS

In this study, we investigated the temporal and spatial resolution of soil eDNA surveys of plant biodiversity to interpret the utility of this approach to effectively assess biodiversity and monitor vegetation changes through time and space. Our results show that a combination of aboveground vegetation surveys and soil eDNA surveys yields the most comprehensive inventory of plant diversity for a site. In particular, a single soil eDNA sample mainly detects local plant diversity rather than site specific diversity. In addition, a soil eDNA sample captures plant diversity going back at least 30 years in time while matching most closely with current diversity. Similarly, we find that soil eDNA samples can be useful to detect both rare and unrecorded taxa, but are best at detecting abundant taxa. Our results highlight the potential of soil eDNA surveys to monitor vegetation responses over broader spatial and temporal scales, and encourage a rethinking of the optimal strategies for assessment of vegetation if soil eDNA is used as a method.

ACKNOWLEDGEMENTS

Morphological surveys were conducted as a part of a project from the Natural History Museum, University of Oslo. We recognise the great effort put into these surveys by their participants over 30 years. We like to thank Andreas Wollan for his support during soil sampling and plot recognition in the Solhomfjell Forest Reserve, Jarl Andreas Anmarkrud, Lisbeth Torbek and Audun Schrøder-Nielsen for their support in the laboratory for DNA extraction, library building and sequencing, Youri Lammers for his support in metabarcoding bioinformatics, Marcella Orwick Rydmark for her support and valuable feedback in manuscript writing, Rebecca Angelica Blakeney for proof-reading the manuscript. This research is part of the H2020 MSCA-ITN-ETN Plant.ID network, and has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 765000.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

M.A., I.G.A., R.H. and H.J.d.B. conceived the study and methodology; M.A. collected the data and built the metabarcoding libraries in the laboratory; M.A. analysed the data with scientific and coding advice from I.G.A., R.H., and B.F., Q.M., respectively; M.A. and H.J.d.B. drafted the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/2041-210X.13865.

DATA AVAILABILITY STATEMENT

Raw sequences from both MiSeq runs (F and R) and bash and R codes are available in Ariza et al. (2022).

ORCID

María Ariza https://orcid.org/0000-0001-8396-4902
Bertrand Fouks https://orcid.org/0000-0002-3675-3499
Quentin Mauvisseau https://orcid.org/0000-0003-1215-2987
Rune Halvorsen https://orcid.org/0000-0002-6859-7726
Inger Greve Alsos https://orcid.org/0000-0002-8610-1085
Hugo J. de Boer https://orcid.org/0000-0003-1985-7859

REFERENCES

Alsos, I. G., Lammers, Y., Yoccoz, N. G., Joergensen, T., Sjoegren, P., Gielly, L., Edwards, M. E., Alsos, I. G., Lammers, Y., Yoccoz, N. G.,

Jørgensen, T., Sjögren, P., Gielly, L., & Edwards, M. E. (2018). Plant DNA metabarcoding of lake sediments: How does it represent the contemporary vegetation. *PLOS ONE*, *13*(4), e0195403. https://doi.org/10.1371/journal.pone.0195403

Alsos, I. G., Lavergne, S., Merkel, M. K. F., Boleda, M., Lammers, Y., Alberti, A., Pouchon, C., Denoeud, F., Pitelkova, I., Puşcaş, M., Roquet, C., Hurdu, B.-I., Thuiller, W., Zimmermann, N. E., Hollingsworth, P. M., & Coissac, E. (2020). The treasure vault can be opened: Large-scale genome skimming works well using herbarium and silica gel dried material. *Plants*, *9*(4), 432. https://doi.org/10.3390/plants9040432

Amend, A. S., Seifert, K. A., & Bruns, T. D. (2010). Quantifying microbial communities with 454 pyrosequencing: Does read abundance count? *Molecular Ecology*, *19*(24), 5555–5565. https://doi.org/10.1111/j.1365-294X.2010.04898.x

Anglès d'Auriac, M. B., Strand, D. A., Mjelde, M., Demars, B. O. L., & Thaulow, J. (2019). Detection of an invasive aquatic plant in natural water bodies using environmental DNA. *PLoS ONE*, *14*(7), e0219700. https://doi.org/10.1371/journal.pone.0219700

Ariza, M., Fouks, B., Mauvisseau, Q., Halvorsen, R., Alsos, I.G. & de Boer, J. H. (2022). Using soil eDNA for plant diversity assessments. Zenodo, https://doi.org/10.5281/zenodo.6400498

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation*, *29*(7), 2089–2121. https://doi.org/10.1007/s10531-020-01980-0

Bergamini, A., Pauli, D., Peintinger, M., & Schmid, B. (2001). Relationships between productivity, number of shoots and number of species in bryophytes and vascular plants. *Journal of Ecology*, *89*(6), 920–929. https://doi.org/10.1111/j.1365-2745.2001.00613.x

Bertrand, R., Lenoir, J., Piedallu, C., Riofrío-Dillon, G., de Ruffray, P., Vidal, C., Pierrat, J.-C., & Gégout, J.-C. (2011). Changes in plant community composition lag behind climate warming in lowland forests. *Nature*, *479*(7374), 517–520. https://doi.org/10.1038/nature10548

Boukhdoud, L., Saliba, C., Parker, L. D., Rotzel McInerney, N., Ishak Mouawad, G., Kharrat, M., Kahale, R., Chahine, T., Maldonado, J. E., & Bou Dagher-Kharrat, M. (2021). First DNA sequence reference library for mammals and plants of the Eastern Mediterranean Region. *Genome*, *64*(1), 39–49. https://doi.org/10.1139/gen-2019-0194

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: Aunix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176–182. https://doi.org/10.1111/1755-0998.12428

Brunbjerg, A. K., Bruun, H. H., Dalby, L., Fløjgaard, C., Frøslev, T. G., Høye, T. T., Goldberg, I., Læssøe, T., Hansen, M. D. D., Brøndum, L., Skipper, L., Fog, K., & Ejrnæs, R. (2018). Vascular plant species richness and bioindication predict multi-taxon species richness. *Methods in Ecology and Evolution*, *9*(12), 2372–2382. https://doi.org/10.1111/2041-210X.13087

Capo, E., Giguet-Covex, C., Rouillard, A., Nota, K., Heintzman, P. D., Vuillemin, A., Ariztegui, D., Arnaud, F., Belle, S., Bertilsson, S., Bigler, C., Bindler, R., Brown, A. G., Clarke, C. L., Crump, S. E., Debroas, D., Englund, G., Ficetola, G. F., Garner, R. E., … Parducci, L. (2021). Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: Overview and recommendations. *Quaternary*, *4*(1), 6. https://doi.org/10.3390/quat4010006

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Carrasco-Puga, G., Díaz, F. P., Soto, D. C., Hernández-Castro, C., Contreras-López, O., Maldonado, A., Latorre, C., & Gutiérrez, R. A. (2021). Revealing hidden plant diversity in arid environments. *Ecography*, *44*(1), 98–111. https://doi.org/10.1111/ecog.05100

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026. https://doi.org/10.1126/science.1206432

Corona, P., Chirici, G., McRoberts, R. E., Winter, S., & Barbati, A. (2011). Contribution of large-scale forest inventories to biodiversity assessment and monitoring. *Forest Ecology and Management*, *262*(11), 2061–2069. https://doi.org/10.1016/j.foreco.2011.08.044

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., Kartzinel, T. R., & Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, *28*(2), 391–406. https://doi.org/10.1111/mec.14734

Deagle, B. E., Thomas, A. C., Shaffer, A. K., Trites, A. W., & Jarman, S. N. (2013). Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: Which counts count? *Molecular Ecology Resources*, *13*(4), 620–633. https://doi.org/10.1111/1755-0998.12103

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. https://doi.org/10.1111/mec.14350

Deiner, K., Yamanaka, H., & Bernatchez, L. (2021). The future of biodiversity monitoring and conservation utilizing environmental DNA. *Environmental DNA*, *3*(1), 3–7. https://doi.org/10.1002/edn3.178

Edwards, M. E., Alsos, I. G., Yoccoz, N., Coissac, E., Goslar, T., Gielly, L., Haile, J., Langdon, C. T., Tribsch, A., Binney, H. A., von Stedingk, H., & Taberlet, P. (2018). Metabarcoding of modern soil DNA gives a highly local vegetation signal in Svalbard tundra. *The Holocene*, *28*(12), 2006–2016. https://doi.org/10.1177/0959683618798095

Epp, L. S., Boessenkool, S., Bellemain, E. P., Haile, J., Esposito, A., Riaz, T., Erséus, C., Gusarov, V. I., Edwards, M. E., Johnsen, A., Stenøien, H. K., Hassel, K., Kauserud, H., Yoccoz, N. G., Bråthen, K. A., Willerslev, E., Taberlet, P., Coissac, E., & Brochmann, C. (2012). New environmental metabarcodes for analysing soil DNA: Potential for studying past and present ecosystems. *Molecular Ecology*, *21*(8), 1821–1833. https://doi.org/10.1111/j.1365-294X.2012.05537.x

Esseen, P.-A., Ehnström, B., Ericson, L., & Sjöberg, K. (1997). Boreal forests. *Ecological Bulletins*, *46*, 16. Retrieved from http://www.jstor.org/stable/20113207

Fadrosh, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*, *2*(1), 6. https://doi.org/10.1186/2049-2618-2-6

Fahner, N. A., Shokralla, S., Baird, D. J., & Hajibabaei, M. (2016). Large-scale monitoring of plants through environmental DNA metabarcoding of soil: Recovery, resolution, and annotation of four DNA markers. *PLoS ONE*, *11*(6), e0157505. https://doi.org/10.1371/journal.pone.0157505

Felton, A., Petersson, L., Nilsson, O., Witzell, J., Cleary, M., Felton, A. M., Björkman, C., Sang, Å. O., Jonsell, M., Holmström, E., Nilsson, U., Rönnberg, J., Kalén, C., & Lindbladh, M. (2020). The tree species matters: Biodiversity and ecosystem service implications of replacing Scots pine production stands with Norway spruce. *Ambio*, *49*(5), 1035–1049. https://doi.org/10.1007/s13280-019-01259-x

Fløjgaard, C., Frøslev, T. G., Brunbjerg, A. K., Bruun, H. H., Moeslund, J., Hansen, A. J., & Ejrnæs, R. (2019). Predicting provenance of forensic soil samples: Linking soil to ecological habitats by metabarcoding

and supervised classification. *PLoS ONE*, *14*(7), e0202844. https://doi.org/10.1371/journal.pone.0202844

Foucher, A., Evrard, O., Ficetola, G. F., Gielly, L., Poulain, J., Giguet-Covex, C., Laceby, J. P., Salvador-Blanes, S., Cerdan, O., & Poulenard, J. (2020). Persistence of environmental DNA in cultivated soils: Implication of this memory effect for reconstructing the dynamics of land use and cover changes. *Scientific Reports*, *10*(1), 10502. https://doi.org/10.1038/s41598-020-67452-1

Fraser, C. I., Connell, L., Lee, C. K., & Cary, S. C. (2018). Evidence of plant and animal communities at exposed and subglacial (cave) geothermal sites in Antarctica. *Polar Biology*, *41*(3), 417–421. https://doi.org/10.1007/s00300-017-2198-9

Hallett, L. M., Jones, S. K., MacDonald, A. A. M., Jones, M. B., Flynn, D. F. B., Ripplinger, J., Slaughter, P., Gries, C., & Collins, S. L. (2016). codyn: An R package of community dynamics metrics. *Methods in Ecology and Evolution*, *7*(10), 1146–1151. https://doi.org/10.1111/2041-210X.12569

Halvorsen, R., Bratli, H., & Wollan, A. K. (2019). Vegetasjonsundersøkelser av boreal barskog i Solhomfjell 2018. In Norsk institutt for naturforskning (NINA) (Ed.), *Terrestrisk naturovervåking i 2018: Markvegetasjon, epifytter, smågnagere og fugl. Sammenfatning av resultater*. Norsk institutt for naturforskning (NINA).

Halvorsen, R., Skarpaas, O., Bryn, A., Bratli, H., Erikstad, L., Simensen, T., & Lieungh, E. (2020). Towards a systematics of ecodiversity: The EcoSyst framework. *Global Ecology and Biogeography*, *29*(11), 1887–1906. https://doi.org/10.1111/geb.13164

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B. Biological Sciences*, *286*(1915), 20191409. https://doi.org/10.1098/rspb.2019.1409

Hartvig, I., Kosawang, C., Kjær, E. D., & Nielsen, L. R. (2021). Detecting rare terrestrial orchids and associated plant communities from soil samples with eDNA methods. *Biodiversity and Conservation*, *30*(13), 3879–3901. https://doi.org/10.1007/s10531-021-02279-4

Hassel, K., Segreto, R., & Ekrem, T. (2013). Restricted variation in plant barcoding markers limits identification in closely related bryophyte species. *Molecular Ecology Resources*, *13*(6), 1047–1057. https://doi.org/10.1111/1755-0998.12074

Hiiesalu, I., Opik, M., Metsis, M., Lilje, L., Davison, J., Vasar, M., Moora, M., Zobel, M., Wilson, S. D., & Pärtel, M. (2012). Plant species richness belowground: Higher richness and new patterns revealed by next-generation sequencing. *Molecular Ecology*, *21*(8), 2004–2016. https://doi.org/10.1111/j.1365-294X.2011.05390.x

Hollingsworth, P. M., Li, D.-Z., van der Bank, M., & Twyford, A. D. (2016). Telling plant species apart with DNA: From barcodes to genomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1702), 20150338. https://doi.org/10.1098/rstb.2015.0338

Kier, G., Mutke, J., Dinerstein, E., Ricketts, T. H., Küper, W., Kreft, H., & Barthlott, W. (2005). Global patterns of plant diversity and floristic knowledge. *Journal of Biogeography*, *32*(7), 1107–1116. https://doi.org/10.1111/j.1365-2699.2005.01272.x

Kistler, L., Ware, R., Smith, O., Collins, M., & Allaby, R. G. (2017). A new model for ancient DNA decay based on paleogenomic meta-analysis. *Nucleic Acids Research*, *45*(11), 6310–6320. https://doi.org/10.1093/nar/gkx361

Kumpula, K. (2020). *Systematic comparison of the relative accuracy of vegetation surveys and soil DNA metabarcoding: Assessing plant biodiversity at different spatial scales* (Master thesis). UMEA University. Retrieved from http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-172130

Lang, A. S., Kruijer, J. D., & Stech, M. (2014). DNA barcoding of Arctic bryophytes: An example from the moss genus Dicranum (Dicranaceae, Bryophyta). *Polar Biology*, *37*(8), 1157–1169. https://doi.org/10.1007/s00300-014-1509-7

Liu, Y., Yan, H.-F., Cao, T., & Ge, X.-J. (2010). Evaluation of 10 plant barcodes in Bryophyta (Mosses). *Journal of Systematics and Evolution*, *48*(1), 36–46. https://doi.org/10.1111/j.1759-6831.2009.00063.x

Matsuhashi, S., Doi, H., Fujiwara, A., Watanabe, S., & Minamoto, T. (2016). Evaluation of the environmental DNA method for estimating distribution and biomass of submerged aquatic plants. *PLoS ONE*, *11*(6), e0156217. https://doi.org/10.1371/journal.pone.0156217

Mauvisseau, Q., Harper, L., Sander, M., Hanner, R. H., Kleyer, H., & Deiner, K. (2021). *The multiple states of environmental DNA and what is known about their persistence in aquatic environments*. Authorea, Inc. https://doi.org/10.22541/au.163638394.41572509/v1

Nagler, M., Insam, H., Pietramellara, G., & Ascher-Jenull, J. (2018). Extracellular DNA in natural environments: Features, relevance and applications. *Applied Microbiology and Biotechnology*, *102*(15), 6343–6356. https://doi.org/10.1007/s00253-018-9120-4

Nilsson, M.-C., & Wardle, D. A. (2005). Understory vegetation as a forest ecosystem driver: Evidence from the northern Swedish boreal forest. *Frontiers in Ecology and the Environment*, *3*(8), 421–428. https://doi.org/10.1890/1540-9295(2005)003[0421:UVAAFE]2.0.CO;2

Økland, R. H. & Eilertsen, O. (1993). *Vegetation-environment relationships of boreal coniferous forests in the Solhomfjellarea, Gjerstad, S Norway*. (No. 16; Sommerfeltia, pp. 1–254.). Botanical garden and museum. University of Oslo.

Økland, R. H., & Eilertsen, O. (1994). Canonical Correspondence Analysis with variation partitioning: Some comments and an application. *Journal of Vegetation Science*, *5*(1), 117–126. https://doi.org/10.2307/3235645

Økland, R. H., & Eilertsen, O. (1996). Dynamics of understory vegetation in an old-growth boreal coniferous forest, 1988–1993. *Journal of Vegetation Science*, *7*(5), 747–762. https://doi.org/10.2307/3236386

Økland, T., Bakkestuen, V., Økland, R. H., & Eilertsen, O. (2004). Changes in forest understorey vegetation in Norway related to long-term soil acidification and climatic change. *Journal of Vegetation Science*, *15*(4), 437–448. https://doi.org/10.1111/j.1654-1103.2004.tb02282.x

Osathanunkul, M., Sawongta, N., Pheera, W., Pechlivanis, N., Psomopoulos, F., & Madesis, P. (2021). Exploring plant diversity through soil DNA in Thai national parks for influencing land reform and agriculture planning. *PeerJ*, *9*, e11753. https://doi.org/10.7717/peerj.11753

Palacios, M., Curd, E., Edalati, K., Renshaw, M. A., Dunn, R., Potter, D., Fraga, N., Moore, J., Saiz, J., Wayne, R., & Parker, S. S. (2021). The utility of environmental DNA from sediment and water samples for recovery of observed plant and animal species from four Mojave Desert springs. *Environmental DNA*, *3*(1), 214–230. https://doi.org/10.1002/edn3.161

Parducci, L., Bennett, K. D., Ficetola, G. F., Alsos, I. G., Suyama, Y., Wood, J. R., & Pedersen, M. W. (2017). Ancient plant DNA in lake sediments. *The New Phytologist*, *214*(3), 924–942. https://doi.org/10.1111/nph.14470

Pärtel, M., Hiiesalu, I., Öpik, M., & Wilson, S. D. (2012). Below-ground plant species richness: New insights from DNA-based methods. *Functional Ecology*, *26*(4), 775–782. https://doi.org/10.1111/j.1365-2435.2012.02004.x

Pietramellara, G., Ascher, J., Borgogni, F., Ceccherini, M. T., Guerri, G., & Nannipieri, P. (2009). Extracellular DNA in soil and sediment: Fate and ecological relevance. *Biology and Fertility of Soils*, *45*(3), 219–235. https://doi.org/10.1007/s00374-008-0345-8

Porté, A. J., Samalens, J.-C., Dulhoste, R., Teissier Du Cros, R., Bosc, A., & Meredieu, C. (2009). Using cover measurements to estimate aboveground understorey biomass in Maritime pine stands. *Annales Des Sciences Forestières*, *66*(3), 307. https://doi.org/10.1051/forest/2009005

Prosser, C. M., & Hedgpeth, B. M. (2018). Effects of bioturbation on environmental DNA migration through soil media. *PLoS ONE*, *13*(4), e0196430. https://doi.org/10.1371/journal.pone.0196430

Qi, Y., Wei, W., Chen, C., & Chen, L. (2019). Plant root-shoot biomass allocation over diverse biomes: A global synthesis. *Global Ecology and Conservation*, *18*, e00606. https://doi.org/10.1016/j.gecco.2019.e00606

R Core Team. (2019). *R: A language and environment for statistical computing* (1.2.5001) [Computer software]. R Foundation for Statistical Computing.

Rodriguez-Ezpeleta, N., Morissette, O., Bean, C. W., Manu, S., Banerjee, P., Lacoursière-Roussel, A., Beng, K. C., Alter, S. E., Roger, F., Holman, L. E., Stewart, K. A., Monaghan, M. T., Mauvisseau, Q., Mirimin, L., Wangensteen, O. S., Antognazza, C. M., Helyar, S. J., de Boer, H., Monchamp, M.-E., … Deiner, K. (2021). Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: Comment on 'Environmental DNA: What's behind the term?' by Pawlowski et al. (2020). *Molecular Ecology*, *30*(19), 4601–4605. https://doi.org/10.1111/mec.15942

Rucińska, A., Świerszcz, S., Nobis, M., Zubek, S., Boczkowska, M., Olszak, M., Kosiński, J. G., Nowak, S., & Nowak, A. (2022). Is it possible to understand a book missing a quarter of the letters? Unveiling the belowground species richness of grasslands. *Agriculture, Ecosystems & Environment*, *324*, 107683. https://doi.org/10.1016/j.agee.2021.107683

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sepp, S.-K., Davison, J., Moora, M., Neuenkamp, L., Oja, J., Roslin, T., Vasar, M., Öpik, M., & Zobel, M. (2021). Woody encroachment in grassland elicits complex changes in the functional structure of above- and belowground biota. *Ecosphere*, *12*(5), e03512. https://doi.org/10.1002/ecs2.3512

Soininen, E. M., Gauthier, G., Bilodeau, F., Berteaux, D., Gielly, L., Taberlet, P., Gussarova, G., Bellemain, E., Hassel, K., Stenøien, H. K., Epp, L., Schrøder-Nielsen, A., Brochmann, C., & Yoccoz, N. G. (2015). Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS ONE*, *10*(1), e0115335. https://doi.org/10.1371/journal.pone.0115335

Soininen, E. M., Zinger, L., Gielly, L., Yoccoz, N. G., Henden, J.-A., & Ims, R. A. (2017). Not only mosses: Lemming winter diets as described by DNA metabarcoding. *Polar Biology*, *40*(10), 2097–2103. https://doi.org/10.1007/s00300-017-2114-3

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The principles and practices of statistics in biological research* (3rd ed., p. 880). W. H. Freeman.

Sønstebø, J. H., Gielly, L., Brysting, A. K., Elven, R., Edwards, M., Haile, J., Willerslev, E., Coissac, E., Rioux, D., Sannier, J., Taberlet, P., & Brochmann, C. (2010). Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular Ecology Resources*, *10*(6), 1009–1018. https://doi.org/10.1111/j.1755-0998.2010.02855.x

Steinbauer, M. J., Grytnes, J.-A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H., Rixen, C., Winkler, M., Bardy-Durchhalter, M., Barni, E., Bjorkman, A. D., Breiner, F. T., Burg, S., Czortek, P., Dawes, M. A., Delimat, A., Dullinger, S., Erschbamer, B., Felde, V. A., … Wipf, S. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature*, *556*(7700), 231–234. https://doi.org/10.1038/s41586-018-0005-6

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, *21*(8), 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., & Willerslev, E. (2007). Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research*, *35*(3), e14. https://doi.org/10.1093/nar/gkl938

Terwayet Bayouli, I., Terwayet Bayouli, H., Dell'Oca, A., Meers, E., & Sun, J. (2021). Ecological indicators and bioindicator plant species for biomonitoring industrial pollution: Eco-based environmental assessment. *Ecological Indicators*, *125*, 107508. https://doi.org/10.1016/j.ecolind.2021.107508

Träger, S., Öpik, M., Vasar, M., & Wilson, S. D. (2019). Belowground plant parts are crucial for comprehensively estimating total plant richness in herbaceous and woody habitats. *Ecology*, *100*(2), e02575. https://doi.org/10.1002/ecy.2575

Uuemaa, E., Mander, Ü., & Marja, R. (2013). Trends in the use of landscape spatial metrics as landscape indicators: A review. *Ecological Indicators*, *28*, 100–106. https://doi.org/10.1016/j.ecolind.2012.07.018

Wang, Y., Pedersen, M. W., Alsos, I. G., De Sanctis, B., Racimo, F., Prohaska, A., Coissac, E., Owens, H. L., Merkel, M. K. F., Fernandez-Guerra, A., Rouillard, A., Lammers, Y., Alberti, A., Denoeud, F., Money, D., Ruter, A. H., McColl, H., Larsen, N. K., Cherezova, A. A., … Willerslev, E. (2021). Late quaternary dynamics of Arctic biota from ancient environmental genomics. *Nature*, *600*(7887), 86–92. https://doi.org/10.1038/s41586-021-04016-x

Wiens, J. J. (2016). Climate-related local extinctions are already widespread among plant and animal species. *PLoS Biology*, *14*(12), e2001104. https://doi.org/10.1371/journal.pbio.2001104

Willerslev, E., Davison, J., Moora, M., Zobel, M., Coissac, E., Edwards, M. E., Lorenzen, E. D., Vestergård, M., Gussarova, G., Haile, J., Craine, J., Gielly, L., Boessenkool, S., Epp, L. S., Pearman, P. B., Cheddadi, R., Murray, D., Bråthen, K. A., Yoccoz, N., … Taberlet, P. (2014). Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature*, *506*(7486), 47–51. https://doi.org/10.1038/nature12921

Wilson, J. B. (2011). Cover plus: Ways of measuring plant canopies and the terms used for them. *Journal of Vegetation Science*, *22*(2), 197–206. https://doi.org/10.1111/j.1654-1103.2010.01238.x

Yoccoz, N. G. (2012). The future of environmental DNA in ecology. *Molecular Ecology*, *21*(8), 2031–2038. https://doi.org/10.1111/j.1365-294X.2012.05505.x

Yoccoz, N. G., Bråthen, K. A., Gielly, L., Haile, J., Edwards, M. E., Goslar, T., Von Stedingk, H., Brysting, A. K., Coissac, E., Pompanon, F., Sønstebø, J. H., Miquel, C., Valentini, A., De Bello, F., Chave, J., Thuiller, W., Wincker, P., Cruaud, C., Gavory, F., … Taberlet, P. (2012). DNA from soil mirrors plant taxonomic and growth form diversity. *Molecular Ecology*, *21*(15), 3647–3655. https://doi.org/10.1111/j.1365-294X.2012.05545.x

Zinger, L., Taberlet, P., Schimann, H., Bonin, A., Boyer, F., De Barba, M., Gaucher, P., Gielly, L., Giguet-Covex, C., Iribar, A., Réjou-Méchain, M., Rayé, G., Rioux, D., Schilling, V., Tymen, B., Viers, J., Zouiten, C., Thuiller, W., Coissac, E., & Chave, J. (2019). Body size determines soil community assembly in a tropical forest. *Molecular Ecology*, *28*(3), 528–543. https://doi.org/10.1111/mec.14919

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Ariza, M., Fouks, B., Mauvisseau, Q., Halvorsen, R., Alsos, I. G. & de Boer, H. J. (2022). Plant biodiversity assessment through soil eDNA reflects temporal and local diversity. *Methods in Ecology and Evolution*, *00*, 1–16. https://doi.org/10.1111/2041-210X.13865