Ecological Indicators 101 (2019) 313–323

Contents lists available at ScienceDirect

Ecological Indicators

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

Original Articles

The devil is in the detail: Metabarcoding of arthropods provides a sensitive measure of biodiversity response to forest stand composition compared with surrogate measures of biodiversity

N. Barsoum[a,*], Catharine Bruce[b], Jack Forster[a], Yin-Qiu Ji[c], Douglas W. Yu[d]

[a] Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom
[b] Nature Metrics CABI Site, Bakeham Lane, Egham, Surrey, TW20 9TY, United Kingdom
[c] State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
[d] School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk NR47TJ, United Kingdom

ARTICLE INFO

Keywords:
DNA metabarcoding
Malaise traps
Surrogate measures of biodiversity
Biodiversity indicators
Forest management
Tree identity

ABSTRACT

Gauging trends in forest biodiversity and relating these to forest management practice and environmental change requires effective monitoring and assessment of spatio-temporal trends in forest biodiversity. Taxa- and habitat-based surrogate measures of biodiversity, or 'biodiversity indicators', are commonly used to convey information about the state of the biological community since they can be assessed relatively quickly and cheaply by non-experts. Direct measures of a component of biodiversity are also increasingly feasible using DNA metabarcoding; 'Next Generation Sequencing' has facilitated the rapid characterisation of combined multiple species samples by sequencing their DNA barcodes in parallel, simultaneously reducing the need for taxonomic expertise and the time and cost required to obtain biodiversity data across a wide range of taxonomic groups.

We investigated whether biodiversity information obtained from DNA metabarcoding of mass-trapped arthropods and from a range of taxa-based surrogate measures of biodiversity (e.g. carabid beetles, vascular plants) provide: 1) similar estimates of alpha and beta diversity and 2) provide similar forest management related conclusions. We also explored how well habitat-based surrogate measures of biodiversity (e.g. stand structure, volume of deadwood) predict observed biodiversity patterns. The study was conducted in Thetford Forest, UK within 15 forest plantation stands (5 Scots pine-oak mixtures, 4 Scots pine and 6 oak monocultures).

Our results demonstrated a high level of congruence between the metabarcoding and taxa-based surrogate measures of biodiversity. The wider range of taxonomic groups identified using a metabarcoding approach offered the potential to identify taxa sensitive to the environmental variable that was being manipulated experimentally (i.e. the composition of forest stands). Most habitat-based measures of biodiversity failed to predict species assemblage differences between stands.

1. Introduction

In recent decades there has been a growing recognition that forest management needs to balance the profitability of forest products against negative impacts on biodiversity and associated woodland ecosystem functioning and resilience (Paquette and Messier, 2010; Puettmann, 2011; Verheyen et al., 2015; Isbell et al., 2017). It is also now widely believed that with appropriate planning and management, production woodlands can play an important role in protecting and enhancing native forest biodiversity (Hartley, 2002; Quine and Humphrey, 2003; Brockerhoff et al., 2008; Gardner, 2012).

The 1992 Convention on Biological Diversity (CBD) provides a legal framework for the conservation of biodiversity and the sustainable use of its components. In the forestry sector, this stimulated the formulation of a suite of Sustainable Forest Management (SFM) principles and guidelines. These included criteria and indicators used to define SFM, but also to measure and report on progress towards the implementation of SFM (McDonald and Lane, 2004; MacDicken et al., 2015). Reflecting these catalysts of change in forest management practice, is an increasing requirement to monitor spatio-temporal trends in forest biodiversity. For example, National Forest Inventories (NFIs) now routinely include, alongside traditional measures of forest

* Corresponding author.
E-mail address: nadia.barsoum@forestry.gsi.gov.uk (N. Barsoum).

https://doi.org/10.1016/j.ecolind.2019.01.023
Received 24 May 2018; Received in revised form 14 December 2018; Accepted 8 January 2019
1470-160X/

productivity, assessments designed to provide biodiversity data for national reporting against set targets to protect and enhance forest biodiversity (Chirici et al., 2012). Biodiversity data is also collected to identify woodlands of conservation interest, to detect threats (e.g. climate change, novel pests and pathogens) to forest biodiversity and to gauge the effectiveness of forest policy measures designed to enhance forest biodiversity. One such policy measure includes 'forest diversification' which can be achieved by fostering polycultures instead of monocultures and creating woodlands with a mixed aged structure (Puettmann, 2011).

There is common agreement among experts of the greater value of 'actual' compared to 'inferred' assessments of biodiversity (Lindenmayer and Likens, 2010; Chirici et al., 2012). Direct assessments of levels of biodiversity are, however, not straightforward. Biodiversity is broad, multidimensional, and multiscale in character making it highly challenging to monitor changes across space and time (Puumalainen et al. 2003; Boutin et al. 2009). To census biodiversity fully, even at the smallest spatial and temporal scales, is often a prohibitively expensive and difficult task. The most common unit of taxonomic enquiry is that of the species (Hajibabaei et al., 2016) but, even at this level, biodiversity monitoring encounters numerous challenges, including: 1) the difficulty and expense of collecting representative samples of species present (e.g. trapping of rare or elusive species), 2) a shortage of taxonomic expertise to identify specimens correctly from their morphology, 3) slow processing of often very large numbers of specimens, resulting in inevitable high related costs and 4) difficulties in identifying species due to poor quality samples, or the presence of juvenile life stages. Thus, biodiversity monitoring has tended to focus on a restricted number of species that are considered to be at risk of extinction, or species that are relatively easy to sample and that are taxonomically unambiguous and therefore easy to identify.

Alternatively, biodiversity monitoring commonly applies surrogate measures of biodiversity, or 'biodiversity indicators' that convey information about the wider state of the biological community and which can be assessed relatively quickly and cheaply by non-experts (Ferris and Humphrey, 1999; Noss, 1999; Coote et al., 2013). There are two categories of commonly used surrogates: taxa-based surrogates (compositional indicators) and habitat-based surrogates (structural indicators). Taxa-based surrogates refer to key taxa that are considered representative of a broader segment of biodiversity (i.e. biodiversity patterns observed for the surrogate taxon are generalizable to one or more taxa) (Sabatini et al., 2016). For example, carabid beetles (Coleoptera: Carabidae), hoverflies (Diptera: Syrphidae), spiders (Araneae), vascular plants and bryophytes are commonly cited as being potentially informative indicators of the species richness of other taxa in forest settings (Ferris and Humphrey, 1999; Cardoso et al., 2004; Pawson et al., 2011; Foord et al., 2013; Gao et al., 2015).

Habitat-based surrogates comprise aspects of the habitat that are thought to affect – and therefore predict- the richness, composition and/or diversity of one of more taxa. Examples of habitat-based surrogate measures of forest biodiversity include volumes of deadwood, levels of canopy cover and woodland stand age and structural complexity; all of these show either positive or negative correlations with species richness, depending on the taxonomic group in question (Gao et al., 2015; Tews et al., 2004). Because of the relative ease of assessing habitat-based surrogates, many of these are now included in NFIs as internationally recognised indicators of SFM and as a primary source of forest biodiversity monitoring data at the national scale (Chirici et al., 2012).

The widespread use of surrogate measures of biodiversity is, nevertheless, revealing some important limitations of these methods for forest biodiversity assessments and monitoring. Gaspar et al. (2010) cautioned that surrogate measures of biodiversity may show different strengths of correlation depending on the geographic scale of inquiry. A recent review has similarly revealed only limited evidence of the universal applicability of many commonly used surrogate measures of biodiversity in different forest ecosystems (Gao et al., 2015). This is because many have not been tested widely across different forest types and in different bioclimatic zones (Cantarello and Newton, 2008). For certain surrogate measures of biodiversity such as volume of deadwood, attempts have been made to set evidence-based threshold levels for biodiversity gains (Humphrey and Bailey, 2012), although there is the complication that these thresholds may need to be adjusted according to regional levels of soil fertility, the bioclimatic zone, or depending on tree species present (Larrieu and Gonin, 2008). Furthermore, to reduce the chances of making incorrect management decisions based on weak or ineffective surrogates that may be biased in favour of a single taxon, several authors now recommend conducting assessments of multiple taxonomic groups, particularly where taxonomic responses to a given environmental variable (e.g. canopy cover) are unknown (Sabatini et al., 2016; Larrieu et al., 2018). While this comprises a considerable sampling and sample identification effort, recent advances in molecular ecology, and DNA metabarcoding in particular, are promising to make this more achievable.

DNA metabarcoding is a powerful species identification method that uses 'next generation sequencing' (NGS) technology to scale up the traditional DNA barcoding process. This allows the rapid characterisation of complex samples of multiple species by sequencing their DNA barcodes in parallel, simultaneously reducing the need for taxonomic expertise and the time and cost required to obtain high quality biodiversity data, across a wide range of taxonomic groups, at large spatial and temporal scales (Yu et al., 2012; Barsoum et al., 2018). Previous studies have shown that metabarcoding arthropods generates accurate and reliable alpha and beta biodiversity information at a fraction of the time and cost of traditional survey methods (Yu et al., 2012; Ji et al., 2013; Morinière et al., 2016).

Here, we explore the potential to apply a metabarcoding approach to measure biodiversity response to subtle differences in forest environmental conditions and we compare this approach with the use of taxa- and habitat-based surrogate measures of biodiversity. Specifically, we investigate the scope for a metabarcoding approach to provide data that can be used to: (1) detect any fine-scale spatial and temporal variation in arthropod community composition in response to tree species composition in plantation forest stands, (2) evaluate the biodiversity effects of different forest management strategies; i.e. plantation monocultures compared with polycultures and (3) identify which species or species groups of arthropods captured in malaise traps are most sensitive to the composition of forest stands. We use a sampling method that is effective at trapping insects from the orders Diptera and Hymenoptera (Matthews and Matthews, 1971; Geiger et al., 2016; Morinière et al., 2016). Despite being among the most species rich groups of arthropods, Diptera and Hymenoptera are almost always overlooked in biodiversity studies because of the difficulty associated with sorting and identifying the inevitably large number of specimens which tend to be characterised by small body size (Jukes and Peace, 2003; Fraser et al., 2008; Geiger et al., 2016).

We posed the following research questions:

(1) In forest stands of differing tree species composition, how does the information obtained from metabarcoding and from taxa-based surrogate measures of biodiversity compare? Do datasets derived from these measures of biodiversity provide similar estimates of alpha and beta diversity, thus providing similar conclusions? Taxa-based surrogate measures of biodiversity used in this study and identified based on morphology, include carabid beetles, spiders, vascular plants and bryophytes.
(2) How well do habitat-based surrogate measures of biodiversity commonly used in NFI's (e.g. stand structure, deadwood volume) predict biodiversity patterns observed by metabarcoding and taxa-based surrogate measures of biodiversity?

Table 1
Summary characteristics of 15 study stands in the Thetford Forest region.

Site code	Site history[+] Landcover 1905–1910	Current stand type[*] (% Pine)	Planting year	Stand Area (ha)	Altitude (m a.s.l.)	Soil type
M1	C/B mix	OK/SP (20)	1941	4.9	25	Brown Earth
M2	C/B mix	OK/SP (74)	1932	3.4	15	Brown Earth
M4	Bare	OK/SP (40)	1934	4.5	30	Brown Earth
M5	Bare	OK/SP (45)	1932	5.2	40	Brown Earth
M6	Bare	OK/SP (24)	1935	5.2	40	Ground Water Gley
O1	Bare	OK (0)	1954	4.7	10	Loamy Texture
O2	Bare	OK (0)	1934	4.9	25	Calcareous Brown Earth
O3	Bare	OK (0)	1934	2.4	35	Brown Earth
O4	Bare	OK (0)	1933	2.9	20	Brown Earth
O5	Bare	OK (0)	1932	6.8	40	Brown Earth
O6	C/B mix	OK (3)	1934	5.2	20	Calcareous Brown Earth
P1	Bare	SP (100)	1930	1.7	30	Brown Earth
P2	Bare	SP (100)	1941	1.6	30	Typical Podzol
P3	C/B mix	SP (100)	1967	3.6	30	Brown Earth
P4	Bare	SP (100)	1937	7.1	35	Calcareous Brown Earth

[+] Land cover classes include conifer woodland (C), broadleaf woodland (B), conifer and broadleaf mixed woodland (C/B mix) and non-wooded areas (Bare) that could in some cases be areas of heathland.
[*] Three stand types: OK/SP = mixture, OK = oak monoculture, SP = Scots pine monoculture.

2. Methods

2.1. Site selection

Fifteen forest plantation stands of three stand types were selected for study: four were monocultures of Scots pine (*Pinus sylvestris* L.), six were monocultures of pedunculate oak (*Quercus robur* L.) and five were intimate mixtures of Scots pine and pedunculate oak. These were located in Thetford Forest, East Anglia in south-east England ($52^0 30'$ N, $0^0 51'$ E; 10–40 m a.s.l.) (Thetford Forest characteristics given in Methods A1 of the Supplementary Material). The average stand size was 4.3 ha and the majority of stands were planted between 1930 and 1941 (Table 1).

Initial stand selection was based on a number of criteria: minimum stand area of 1.5 ha, planting age of between 1930 and 1940, stands must have an even shape (i.e. long, thin stands with significant edge were avoided), and a stand should occur in close proximity (within the same forest management block) as selected examples of the other two stand types of interest to allow for a number of clusters of the different stand types to be sampled across the Thetford Forest region. A planting age range was selected to confine the study to a single stage of the forest harvest cycle, thus minimising the influence of stand age as a variable. Enough stands were not always found to accommodate these selection criteria, requiring two younger stands to be included (i.e. O1 and P3 planted in 1954 and 1967, respectively). The 15 stands occurred in approximately four clusters 4–12 km apart, each cluster comprising the three different plantation types.

2.2. Data collection

Biodiversity assessments comprised direct measures of biodiversity by sampling: 1) diverse taxonomic groups of flying arthropods and identifying species using metabarcoding techniques to establish the metabarcode (MBC) dataset and 2) a range of commonly used taxa-based surrogate measures of biodiversity (carabid beetles, spiders, vascular plants and bryophytes) identified based on morphology and contributing to the ‘Standard’ (STD) datasets. Indirect measures of biodiversity were also collected using habitat-based surrogate measures of biodiversity commonly used in NFI’s. These included measures of tree species composition, stand stem density and structural complexity and abundance and volume of deadwood.

2.2.1. Diverse arthropod taxa – metabarcode (MBC) dataset

Malaise traps were used to sample sub-canopy flying arthropods. A single malaise trap was erected within a 10 m radius of the centre of each stand in a space equidistant between trees, avoiding stumps, large logs and shrubs. The orientation of the malaise traps was the same in each stand; i.e. northern-most position of the trap was the main pole holding the arthropod collection vessel. Sterile collecting bottles were 2/3 filled with 100% ethanol and replaced with new ones at weekly sampling intervals for eight consecutive weeks from the 8th of August until the 4th of October 2011, giving a total of 120 (8 × 15) malaise trap samples.

2.2.2. Taxa-based surrogate measures of biodiversity – standard (STD) datasets

Eight pitfall traps were used to sample ground-dwelling spiders and carabids in each stand (trap layout details given in Supp. Mat. Methods A2). Trap contents were collected at 7 fortnightly intervals from May to August 2011. The eight pitfall trap samples in each stand were pooled together at each sample interval. Ground-dwelling spiders and carabid beetles were identified morphologically to species level using the keys of Roberts (1993; spiders) and Luff (2007; carabids).

Vascular plants and bryophytes were surveyed in eight 2 × 2-m quadrats in each stand during the first two weeks in July 2011 (quadrat layout details given in Supp. Mat. Methods A2). The percentage cover of each terrestrial (including saxicolous and epixylic) species of vascular plant and bryophyte was estimated using the DOMIN cover-abundance scale in quadrats and the nomenclature of vascular plants and bryophytes followed Stace (2010) and Smith (2004), respectively.

2.2.3. Habitat-based surrogate measures of biodiversity

In February 2013, fourteen of the fifteen stands were surveyed to derive 16 habitat-based surrogate measures of biodiversity listed in Table 2 and described in Methods A3 (Supp. Mat.); stand P2 could not be surveyed because it had been harvested. Definitions and assessments of stem density, deadwood and tree stumps were broadly based on those used in the UK National Forest Inventory (UK NFI, 2016).

2.3. Metabarcode protocols and data preparation

Details of sample preparation, DNA extraction, PCR and sequencing are provided in Supp. Mat. Methods A4. Methods used for the bioinformatic extraction of Operational Taxonomic Units (OTU’s) from raw sequence data are provided in Supp. Mat. Methods A5.

A total of 1123 molecular OTUs were generated, each OTU representing a distinct species. While duplicates of many of these 1123 OTUs occurred, species abundance cannot be reliably inferred from multiple identical OTUs. Quality control filtering included: 1) setting a threshold of > 97% similarity match of OTU sequences, 2) the removal

Table 2
Names and descriptions of habitat-based surrogate measures of biodiversity included in study.

Variable	Description
Tree species	Number of tree species with at least one measurable stem
%Pine	Percentage of measurable stems (crop and non-crop; live and dead) that are Scots pine. A measure of the broadleaf/conifer ratio
Stem density	Number of measurable stems (live and dead) in 900 m^2 block
Crop density	Number of crop stems (i.e. Scots pine and/or oak) in 900 m^2 block
Non-crop density	Number of non-crop stems in 900 m^2 block; i.e. non-canopy Scot spine and/or oak and other tree species present
SCI	Structural complexity index (Zenner and Hibbs, 2000)
ESCI 1	Enhanced SCI, modification step 1 (ESCI'). Incorporates triangle orientations (Beckschäfer et al., 2013)
ESCI 2	Enhanced SCI, modification step 2 (ESCI). Incorporates triangle orientations and stem density (Beckschäfer et al., 2013)
Simpson count	Simpson's diversity index D for trees, based on count of measurable stems
Simpson area	Simpson's diversity index D for trees, based on cross-sectional area of measurable stems
Deadwood area	Total cross-sectional area of lying deadwood stems intersecting transect line
Deadwood count	Number of lying deadwood pieces intersecting transect lines
Stump area	Total cross-sectional area of stumps in circular plots based on stump height and diameter
Stump count	Total number of stumps in circular plots
DS area	Deadwood area + Stump area
DS count	Deadwood count + Stump count

of single-read OTUs and 3) the removal of non-arthropods and any species with no prior record of occurrence in the UK. This reduced the number of OTUs down to 521. Of these, 67% were identifiable to species level, 8% to Genus and the remaining 25% to Order level.

Two primary metabarcode dataframes were created from the 521 OTUs that were generated from the malaise trap samples. These dataframes included a 'binary' dataframe and a 'pooled' dataframe. For the binary data frame, every OTU was scored for presence-absence in each of the 120 malaise trap samples. This dataframe was used for: 1) visualising compositional differences among samples grouped by stand type and by sample collection week (1–8) (beta diversity) and 2) for analysis of arthropod species richness between stand types (alpha diversity). In order to increase the confidence of species occurrence, single occurrence OTUs across the 120 malaise trap samples were removed from the binary dataframe.

For the pooled dataframe, where OTUs occurred in a single replicate stand, these were removed (i.e. even if an OTU was present across all eight weeks, it was excluded if it was present in only a single replicate stand). The pooled dataframe comprised species by stand data, in which the eight weekly samples were pooled within each stand. For each stand, every OTU was assigned a value between 0 and 8, representing the number of weeks in which it was detected. This index is not a direct measure of OTU abundance, but it is expected to represent each species' contribution, over time, to a forest stand's arthropod diversity. This dataset was used: (1) for comparisons with the STD datasets to check for consistency of between stand type trends in species richness and (2) to test for any correlations between habitat-based surrogate measures of biodiversity and beta diversity patterns. To allow for a better comparison with the spider STD dataset, an MBC dataset was created from the pooled dataframe to include only spider OTUs ('Araneae MBC dataset').

2.4. Statistical analyses

All statistical analyses were performed using R 3.3.1 (R Core Team, 2016). The following R packages were predominantly used in the analyses: Base R package (R Core Team, 2016), Package "car" (Fox & Weisberg, 2011) for ANOVA, Package "lme4" (glmer function) (Bates et al., 2015) for Generalised linear (mixed effects) modelling (GLM/GLMM), Package "lmerTest" (Kuznetsova et al., 2014) for GLMM ANOVA, Package "lsmeans" (Lenth, 2015) for post-hoc tests least-square means, Package "mvabund" (Wang et al., 2012; Warton et al., 2012) for multivariate likelihood ratio (LR) tests, Package "multcompView" (Graves et al., 2015) for least-square means lettering and Package "vegan" (Oksanen et al., 2016) for nonmetric multidimensional scaling (NMDS) ordination.

2.4.1. Comparing species richness and community composition between stand types – MBC and STD datasets

2.4.1.1. Species richness between stand types. For the MBC dataset, total species richness per stand type was estimated using the Chao2 incidence coverage method (Chao, 1987; Colwell and Coddington, 1994), using vegan function specpool(), and compared between pairs of stand types using Welch's t-tests. Resulting p-values were adjusted for three pairwise tests.

For the STD datasets, two metrics were used: (i) the total number of species present in each stand (TSR) (i.e. 8 quadrats/pitfall traps combined) and (ii) the mean species richness (S) per 2 × 2-m quadrat/ per pitfall trap. GLMs and GLMMs with log link function and Poisson errors were used to model the effect of the explanatory variable (stand type) on the response variables (TSR, S). For mean species richness, where quadrats/pitfall traps were nested within stands, stand was used as a random effect in the mixed effects models. Since Araneae and Carabid data were collected at six intervals, collection interval was included as a factor and interaction term within the model. Where explanatory variables had a significant effect, post hoc multiple comparisons with Tukey corrections were applied.

2.4.1.2. Community composition between stand types. To visualise stand type influences on community compositions NMDS ordination of Jaccard dissimilarity matrices were created (function metaMDS() in vegan) using the MBC data. Data were displayed to show species richness differences across stand types (functions ordisurf() and ordispider()in vegan).

Multivariate LR tests were used to test for an effect of stand type on community composition across the MBC and STD data sets. In addition to testing for an overall effect of stand type, Post hoc tests were used to make pairwise comparisons between stand types, with p-values adjusted for three pairwise comparisons using Benjamini and Hochberg's (1995) correction method (p.adjust(method = fdr) in R). Further details of the rationale and methods of applying the multivariate LR tests are given in Supp. Mat. Methods A6.

2.4.1.3. Direct comparison of MBC and STD datasets. Quantitative Jaccard distance matrices and NMDS ordinations (function metaMDS () in vegan) were created for each of the STD data sets (i.e. Araneae, Carabidae, bryophytes and vascular plants) and two MBC datasets (all arthropods and Araneae only), thereby preserving OTU frequency information. MBC and STD datasets were subsequently compared using both Procrustes and Mantel tests, each with 999 permutations, as recommended, to assess similarity between ordinations (Forcino et al., 2015).

2.4.2. Comparing habitat-based surrogate measures of biodiversity between stand types and in relation to MBC datasets

Multivariate LR tests were used to test for an effect of each of the habitat-based surrogate measures of biodiversity on community composition across the pooled arthropod MBC data, using Poisson distributions in each case. Likelihood ratio test statistics were used to determine the significance of each variable. For each variable that was significant, OTU-specific p-values and LR coefficients were used to determine the number of OTUs (by arthropod order) that showed the strongest response to the selected habitat-based surrogate measure of biodiversity.

2.4.3. Temporal variations in community composition – MBC dataset

Data were displayed using an NMDS ordibnation to show species richness effects across stands and time (functions ordisurf() and ordispider()in vegan). To explore time effects, data were modelled using the lmer() package in a mixed-effects model. Species richness data included all species present, including those that appeared only once within the binary data frame. Analysis of variance from the lmerTest() package (type III with Satterthwaite approximation for degrees of freedom) was used to determine significant fixed effects using a best fit model for both the MBC and Araneae MBC data. To test for differences in species associated with the first half (weeks 1–4; August) and the second half (weeks 5–8; September) of the sampling period, multivariate LR tests were conducted with binomial errors and 999 bootstrap iterations. Further details of the mixed effects model that was applied and model selection are provided in Supp. Mat. Methods A7.

3. Results

3.1. Comparing species richness and community composition between stand types – MBC and STD datasets

3.1.1. Taxonomic composition of MBC and STD datasets

3.1.1.1. MBC dataset. The 521 OTU's making up the MBC dataset were distributed across four arthropod Classes: Arachnida, Diplopoda, Insecta and Malacostraca. Diptera were a dominant order (65% of all OTUs), followed by Coleoptera (8%), Araneae, Hemiptera, Hymenoptera (each making up 6% of all OTUs) and Lepidoptera (3%) (Table 3 and Supp. Mat. Table A1). Identification of OTU's to species level was lowest among the Hymenoptera (52%) and Diptera (60%) and highest among better known orders such as Lepidoptera (95%), Araneae (83%) and Coleoptera (90%) which have comparatively high numbers of national recordings (NBN Atlas, 2017). Across all stands, a total of 30 spider species were identified from 10 families. Two families of spider were unique to the MBC dataset; these were orb weaver spiders (Araneidae) and mesh web weaver spiders (Dictynidae) that weave webs in vegetation. A single carabid beetle species was identified in the MBC dataset (*Cychrus* sp.). A number of species identified are nationally scarce or are species of declining numbers (e.g. the crab spider, *Xysticus lanio*; the Green-brindled Crescent moth, *Allophyes oxyacanthae*) and some (n = 46) from the Diptera, Hemiptera and Hymenoptera families have never previously been recorded in the Norfolk region (highlighted in Supp. Mat. Table A1). For a number of taxonomic groups (e.g. some fly and gnat families such as the Phoridae, Sciaridae, Ceratopogonidae) many species were detected that have rarely been recorded in the UK. The MBC data also revealed the presence of a potentially important disease vector species, the biting midge *Culicoides scoticus*, which could be an important vector of Bluetongue virus, a serious pathogen of ruminants (Carpenter et al., 2008).

Table 3
Taxonomic composition of MBC dataset.

Class	Order	Number of species/ OTUs	Percentage of total
Arachnida	Araneae	30	5.7
	Opiliones	5	1.0
	Sarcoptiformes	1	0.2
Diplopoda	Julida	1	0.2
Insecta	Coleoptera	39	7.5
	Dermaptera	2	0.4
	Diptera	338	64.8
	Hemiptera	29	5.6
	Hymenoptera	31	5.9
	Lepidoptera	18	3.4
	Mecoptera	3	0.6
	Neuroptera	6	1.2
	Orthoptera	5	1.0
	Plecoptera	1	0.2
	Psocodea	8	1.5
	Psocoptera	1	0.2
	Trichoptera	1	0.2
Malacostraca	Isopoda	2	0.4

3.1.1.2. STD datasets. A total of 86 spider species, belonging to 17 different families, were identified in pitfall trap samples across all stands (Table Supp. Mat. Table A2). Spiders were present from eight families that did not occur in the MBC dataset. Among these were typical ground-dwelling species such as wolf (Lycosidae) and prowling (Miturgidae) spiders. A total of 37 ground-dwelling carabid species were identified from pitfall traps in all stands. Twelve of these species are frequently associated with woodlands as indicated in Supp. Mat. Table A3. A total of 67 vascular plant species and 15 bryophyte species were identified in quadrats (Supp. Mat. Tables A4 and A5, respectively).

3.1.2. Species richness between stand types

3.1.2.1. MBC dataset. No significant differences in estimated total species richness were found between oak monocultures and mixtures of Scots pine and oak, although both of these stand types had significantly higher estimated species richness than Scots pine monocultures (Fig. 1). Although fewer pine monoculture stands were sampled than mixtures of Scots pine and oak, species accumulation curves indicate sufficient sampling effort for all three stand types, with the curve for Scots pine monoculture stands clearly levelling off at a

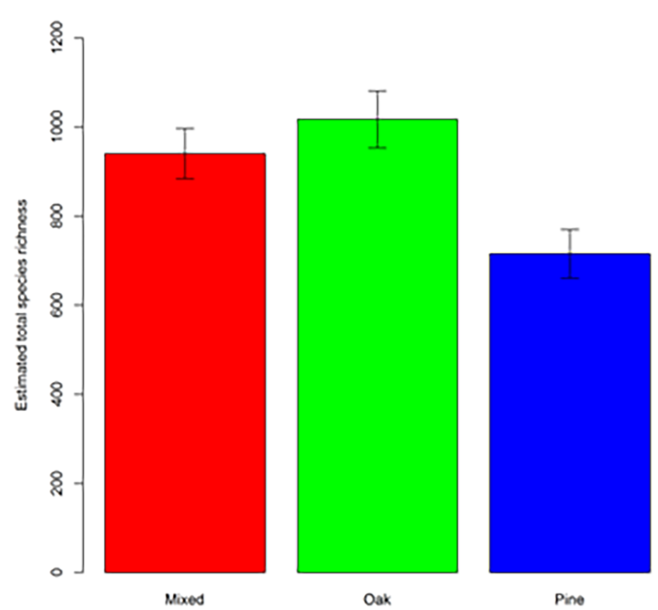

Fig. 1. Estimated extrapolated species richness (alpha diversity) of all arthropods combined (MBC dataset) in Scots pine oak mixed stands, and in oak and Scots pine monocultures calculated using the Chao equation. Error bars indicate standard errors.

lower species richness than those of the other stand types (Supp. Mat. Fig. A1).

3.1.2.2. STD datasets. Of the four STD datasets, only carabid and bryophyte total and mean species richness (TSR and S) showed significant differences between oak and Scots pine monocultures. There were significantly more bryophyte species, but significantly fewer carabid species in Scots pine monocultures compared with oak monocultures (Table A6). For both of these taxonomic groups, species richness in Scots pine-oak mixtures resembled the oak monocultures. In the case of spiders, a significant interaction was detected between stand type and collection interval with spider species richness in Scots pine and oak monocultures differing significantly at only one collection interval.

3.1.3. Community composition between stand types

An NMDS ordination of the MBC dataset showing arthropod samples grouped by stand type, revealed a greater similarity in the species compositions of oak monocultures and Scots pine-oak mixtures compared with Scots pine monocultures (Supp. Mat. Fig. A2). Multivariate likelihood ratio (LR) tests showed significant differences in species composition across the three stand types, with 30 OTUs associated with Scots pine-oak mixtures, 46 OTU's associated with oak monocultures and 40 OTU's associated with pine monocultures. These included species from a wide range of taxonomic Orders, although the majority were Diptera (Supp. Mat. Tables A1 and A7). Conifer-associated species included one potential disease vector: the biting midge *Culicoides scoticus*. The mvabund analysis showed significant differences across the three stand types for the majority of the MBC and STD data sets; pairwise comparisons of stand type are shown in Table 4. Although some of the datasets were not significant at a 0.05 level (likely due to the small sample size), there was a general trend for significant differences to be predominantly driven by pine monocultures compared with the other two stand types. The consistency across MBC and STD data sets provides evidence of consistent results across MBC and STD measures of biodiversity.

3.1.4. Direct comparison of MBC and STD datasets

Fig. 2 (A-F) shows the results of the NMDS ordinations, grouped by stand type, for the MBC (Fig. 2: A & B) and the STD (Fig. 2: C-F) datasets. The data tend to show similar patterns, with pine monocultures being separate from the other two stand types along the primary axis. Comparison of ordinations from the Araneae pooled MBC and STD Araneae, Carabidae and vascular plant data sets indicated that the MBC and STD datasets contain similar diversity information, with significant correlation between the NMDS ordinations and Jaccard distance matrices from the MBC and STD datasets (Table 5). Comparison of ordinations from the total pooled MBC dataset and the bryophyte STD dataset and comparison of the Araneae pooled MBC dataset and the STD Araneae dataset indicated that the MBC and STD datasets may contain similar diversity information, with significant correlation between the NMDS ordinations but not the Jaccard distance matrices from the MBC and STD datasets; this latter lack of correlation may be related to the limited number of spiders identified in the MBC dataset.

Table 4
Results of Multivariate LR tests applied to MBC and STD data sets, comparing each stand type separately. P-values (*p*) are adjusted for three tests using Benjamini and Hochberg's (1995) correction. Significant associations with stand type are shown in bold italics.

Data Set	Overall *p*	Oak *p*	Pine *p*	Mix *p*
Pooled all arthropods MBC	***0.05***	0.23	0.09	0.40
Araneae MBC	***0.03***	0.05	***0.05***	0.63
Pooled pitfall STD	***0.01***	0.09	***0.02***	0.37
Araneae pitfall STD	***0.01***	***0.05***	***0.04***	0.27
Carabidae pitfall STD	***0.02***	0.46	***0.03***	0.47
Bryophyte STD	***0.02***	0.13	***0.02***	0.50
Vascular plants STD	0.12	0.34	0.27	0.27

3.2. Comparing habitat-based surrogate measures of biodiversity between stand types and in relation to MBC datasets

The mvabund analysis showed significant differences across only one of the surrogate variables: percentage of pine cover (community ~ perc_pine (Poisson errors), $Dev_{(1,13)} = 1480$, $p = 0.02$). OTU-specific p-values and LR coefficients were used to determine the number of OTUs (by arthropod order) that showed the strongest response to percentage pine (Table 6), with Diptera and Araneae being the predominant orders showing a response. Fig. 3 shows a heat map plot of the arthropod MBC data arranged by stand type and % of pine within each stand, showing how different taxa are driving community differences between stand types. Sites P2 and P4 feature particularly distinct arthropod communities. These are pure pine monocultures that lack broadleaf trees even in the understory.

3.3. Temporal variations in community composition – MBC dataset

Analysis of variance applied to the mixed effect model indicated no significant effects of stand type or the interaction between stand type and time (days) (Fig. 4). When the same best fit model was applied to Araneae only MBC data, these data would not converge even with the increased number of dimensions. Analysis of the second NMDS dimension by week as a factor*stand type showed significant main effects with no interaction, where week as a response was non-linear (Fig. A3). Splitting the data into two halves (weeks 1 to 4 and weeks 5 to 8) identified 53 OTUs as being strongly associated with the first half of the trapping period and 54 with the second half. The majority of species driving the temporal effect were dipterans, along with several hymenopteran species (Table A8). Associations are consistent with the species biology. For example, the moth species *Tischeria ekebladella* (associated with weeks 1–4) typically flies in the summer, entering a larval stage from September. Similarly, the ant species *Myrmica ruginodis* was detected in several stands during the first three trapping weeks, after which it was never detected; this is consistent with mating flights for this species which occur in July and August.

4. Discussion

4.1. MBC and STD datasets of multiple taxonomic groups show similar alpha and beta diversity trends across different stand types with comparable forest management implications

The MBC and STD datasets both showed a distinctiveness in the composition of communities sampled in Scots pine monocultures compared with oak monocultures for all taxonomic groups assessed. In Scots pine-oak mixed stands, MBC and STD datasets also showed the same tendency for communities to occupy an "intermediate" position in ordinations, with communities partially comprised of component species present in either Scots pine or oak monocultures. These results are in line with a growing number of studies demonstrating the effectiveness of DNA metabarcoding as a method of collecting reliable biodiversity information that can be used to inform management practice and policy (Ji et al., 2013; Deiner et al., 2017; Elbrecht et al., 2017). In this study, the data provides evidence backing current UK forestry policy that advocates a diversification in the composition of forest stands and woodlands for biodiversity gains (FC, 2017). Thetford Forest is dominated by pine and these results suggest that the inclusion of oak stands as part of the wider mosaic of woodland stands would improve

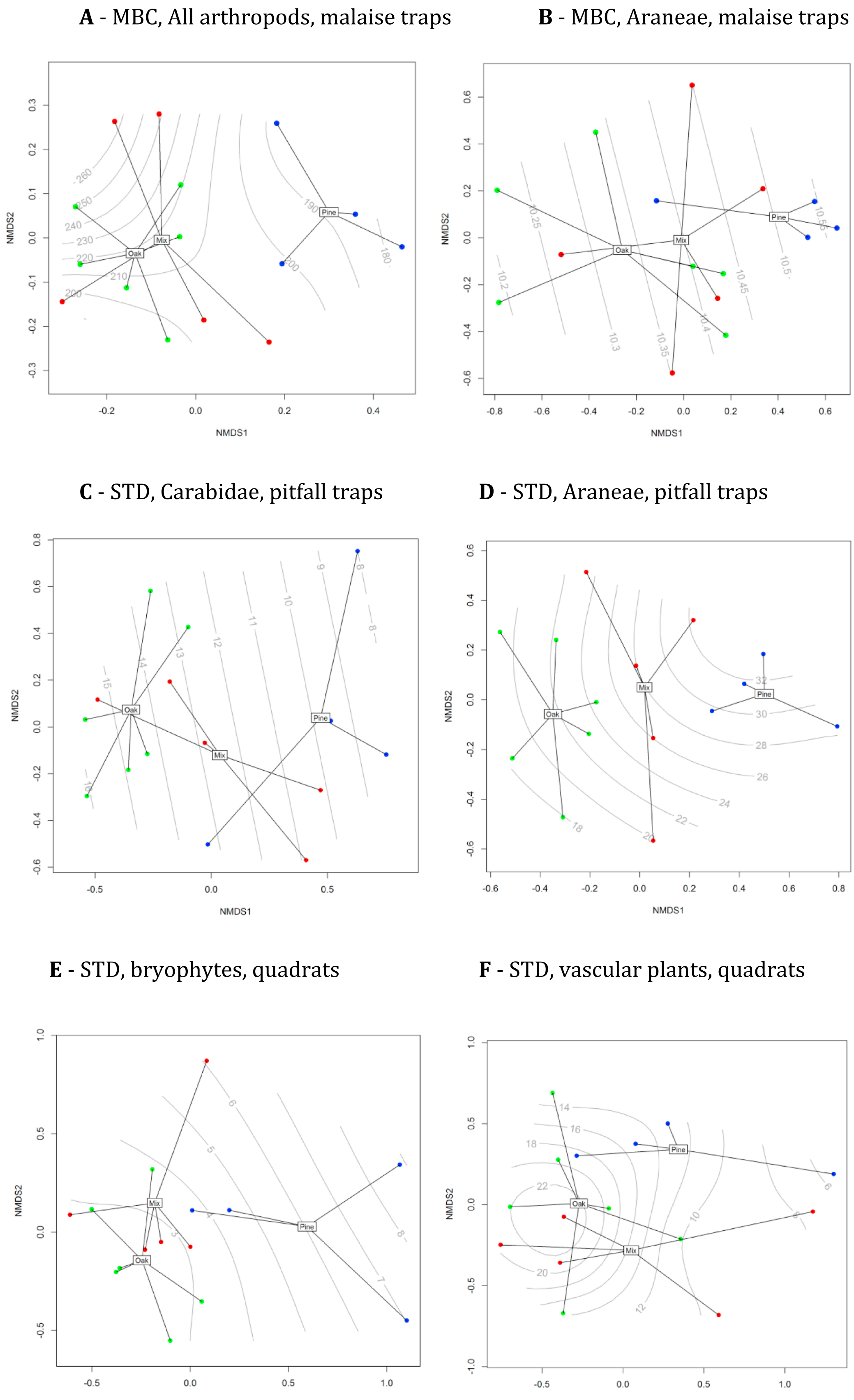

Fig. 2. Non-metric multidimensional scaling (NMDS) ordinations (A-F) of MBC datasets (all arthropods, Araneae only) and STD datasets (spiders, carabids, vascular plants, bryophytes) showing samples grouped by stand type. Surface plot shows species richness.

Table 5
Comparison of MBC and STD datasets; i.e. level of correlation between NMDS ordinations and Jaccard distances matrices.

MBC dataset	STD dataset	Procrustes test correlation	Mantel test r
All arthropods	Araneae	0.68**	0.31**
Araneae	Araneae	0.65**	0.14+
All arthropods	Carabidae	0.58**	0.27*
All arthropods	Bryophytes	0.53*	0.18+
All arthropods	Vascular plants	0.56**	0.30*

Significance level indicated by $^{+}$ < 0.1, * < 0.05, ** < 0.01.

Table 6
Number of OTUs in each taxonomic group that are significantly associated with percentage of pine in a stand.

Order	Number of OTU's associated with % pine
Araneae	9
Opiliones	2
Coleoptera	4
Diptera	39
Hemiptera	2
Hymenoptera	4
Lepidoptera	4
Neuroptera	2
Orthoptera	1
Psocodea	2
Total	**69**

overall levels of alpha and beta diversity. A notable result is the limited ordination space occupied by Scots pine-oak mixtures compared with oak and Scots pine monocultures combined, with mixed stands particularly failing to cover the space occupied by pine monocultures (Fig. 3). This suggests that in oak and Scots pine plantations, improved regional species diversity (for the taxonomic groups considered here) can be achieved by creating a mosaic of pure-oak and pure-pine crops rather than planting intimate mixtures of Scots pine and oak; this is because Scots pine-oak mixtures would incur the loss of pine specialists.

In the Thetford Forest context, Scots pine and oak were clearly favoured by different taxonomic groups; i.e. spiders and bryophytes showed significantly higher species richness in Scots pine monocultures compared with oak monocultures, while carabid beetles showed higher species richness in oak monocultures. There is a need, however, to be cautious about how transferable these taxa-specific responses are in different spatial and temporal contexts. For example, we did not find significant differences in spider species richness between stand types across all sampling intervals. Identical responses have also not been found for many of these taxonomic groups (i.e. vascular plants, spiders, carabids) in other regions of study when comparing these same stand types (Taboada et al., 2010; Barsoum et al., 2016). This inconsistency in taxa-based surrogate measures of biodiversity in different climatic and biogeographical contexts has been reported elsewhere and points to the limitations of focussing biodiversity monitoring and assessment on a single taxa-based surrogate measure of biodiversity, but also over a restricted sampling interval (Kirkman et al., 2012; Sabatini et al., 2016).

4.2. The MBC dataset is more taxonomically comprehensive than STD datasets, allowing for a greater number and range of species associations to be identified by stand type than individual taxa-based surrogate measures of biodiversity

The use of malaise traps and subsequent species identification by metabarcoding allowed for a comparatively large number of species to be sampled across numerous taxonomic groups (particularly among the hyper-diverse Diptera). This improved the chances of identifying whole taxonomic groups that show a particular sensitivity to tree identity, but also individual arthropod species with particular stand type associations; i.e. a total of 116 arthropod species from the MBC dataset had particular stand type associations. For example, high proportions of the dark-winged fungus gnats (Sciaridae) sampled were found to have a significant association to a single stand type. This highlights the scope for the metabarcoding approach to identify taxa-based indicators in forests that demonstrate a particular sensitivity to a given environmental characteristic (e.g. in this case, tree species). It follows that this opens up the possibility of developing and applying metabarcoding as a comparatively rapid and inexpensive tool for routine monitoring (Morinière et al., 2016) in a similar way to current achievements in freshwater ecosystems. Freshwater ecologists are striving and making good progress in the use of DNA metabarcoding of macroinvertebrates to monitor instream water quality (Elbrecht et al., 2017). While species level identification may not be possible for all arthropod specimens sampled due to biases introduced by primers used and reference barcode library limitations, the range and number of arthropod species that can be identified using a metabarcoding approach are nevertheless highly informative and are increasing all the time. Molecular methods have already advanced significantly since we completed the molecular work on our study and yet even with the lower resolution we used compared to what is currently achievable with greater sequencing depth, we were to able detect species: 1) of conservation interest (e.g. Green-brindled Crescent moth, *A. oxyacanthae*), 2) that may pose a biosecurity risk (e.g. the biting midge *C. scoticus* as a potential pathogen vector) and 3) that have not previously been recorded in the region of study. Key to building a monitoring platform using metabarcoding, however, will be the need to standardise sampling and analytical methods for directly transferable and comparable biodiversity estimates (Cristescu, 2014). This is especially vital where it is envisioned that DNA-metabarcoding is applied as a monitoring tool for use within legal and regulatory frameworks (Leese et al., 2018). The careful selection of primers is an additional requirement. Since completing our study, Morinière et al. (2016) have published a study comparing the efficiency of different primers using arthropod samples captured in a malaise trap. Primers used in our study were among those tested by Morinière et al. (2016) who found greater efficiency of amplicons using the dgHCO primer (Leray et al., 2013) than the two primers used in our study; i.e. LCO1490 and HCO2198 (Folmer et al., 1994). This may go some way to explain the surprisingly low proportions of Hymenoptera detected in our study and another malaise trap study that also used Folmer's primers (Yu et al., 2012).

4.3. Most habitat-based surrogate measures of biodiversity tested did not predict significant differences in species assemblages between stands

While some difference in structural complexity and deadwood volume were expected between the different stand types based on the differing characteristics of the tree species (Mason and Connolly, 2014; Shorohova and Kapitsa, 2014; Herrmann et al., 2015, Pretzsch, 2017), these differences were not captured by the variables measured in this study. The range of UK-NFI habitat-based surrogate measures of biodiversity that were assessed revealed a consistency in the measured habitat conditions across the different stands and stand types. Stem density, stand structural complexity, levels of deadwood and the number of canopy and sub-canopy tree species were comparable across the stands and thus, were not useful predictors of significant species and compositional differences observed in the MBC and STD datasets between the different stand types. Only one variable was found to reflect the compositional differences in arthropod communities found in the different stand types based on the MBC dataset; that was the percentage of conifer (i.e. Scots pine) as a proportion of all trees present in the stand. These results suggest that a reliance on the habitat-based surrogate measures of biodiversity applied here would have led to

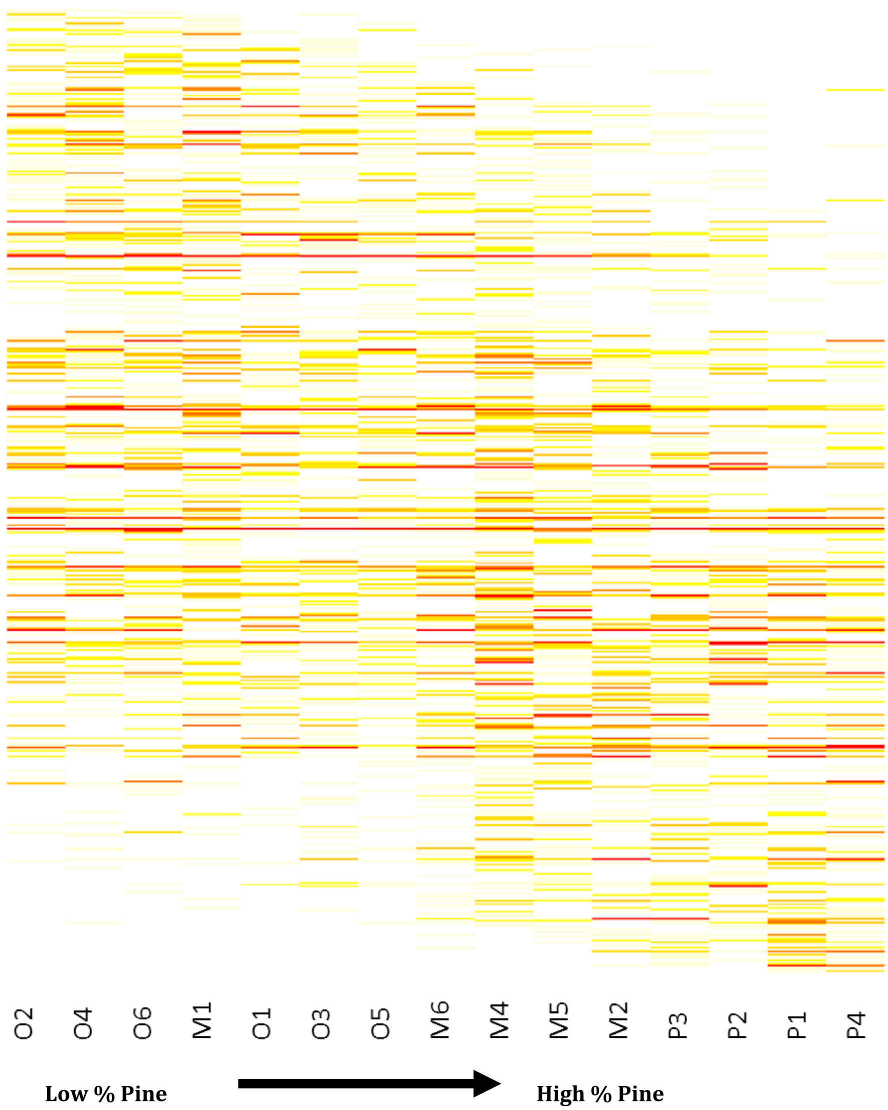

Fig. 3. Pooled total MBC data as a heat map plot. Stands are arranged by percentage of pine present (low to high) on the x-axis. Occurrence of different OTUs are represented by coloured lines on the y-axis.

incorrect assumptions being made about underlying patterns of biodiversity (e.g. significant differences in patterns of species richness between the different forest stand types might have been overlooked).

4.4. Metabarcoding captures fine-scale temporal variations in the composition of arthropod communities

Arthropod sampling can very quickly generate extremely large, unwieldy numbers of specimens, particularly less targeted sampling techniques such as malaise traps. This greatly restricts the number of taxa and repeat samples than can be processed where species identification is based on morphology alone (Humphrey et al., 2003; Morinière et al., 2016). Identification of species using the metabarcoding approach made it possible for a high intensity and frequency of arthropod assemblages to be processed. This provided insight into the very rapid changes in composition of arthropod communities over an eight week period within each stand. Our results showed similar rates of species assemblage change across stands and clear species associations with different sampling periods indicating evident compositional shifts through time. These findings underline the importance of controlling for temporal effects in sampling using malaise traps; this is, particularly true for certain taxonomic groups such as parasitoid wasps since the species composition of samples collected just a couple of weeks apart can differ greatly (Fraser et al., 2008; Geiger et al., 2016). Our findings additionally highlight the potential to relate finely-grained temporal shifts in arthropod communities to fluctuating environmental variables in order to explain the root causes of important shifts in the composition of arthropod communities. This is especially relevant when considering significant reported global declines in the abundance of certain insect groups, including moths, butterflies, bees, spiders and carabid beetles (Hallmann et al., 2017; Leather, 2018). The causal agents of many of these declines are not yet clear, although environmental variables with a negative influence could include levels of air pollution and pesticide use associated with land use intensification, and/or important variations in the seasonality and range of ambient temperatures associated with global warming (Brandon-Mong et al., 2018).

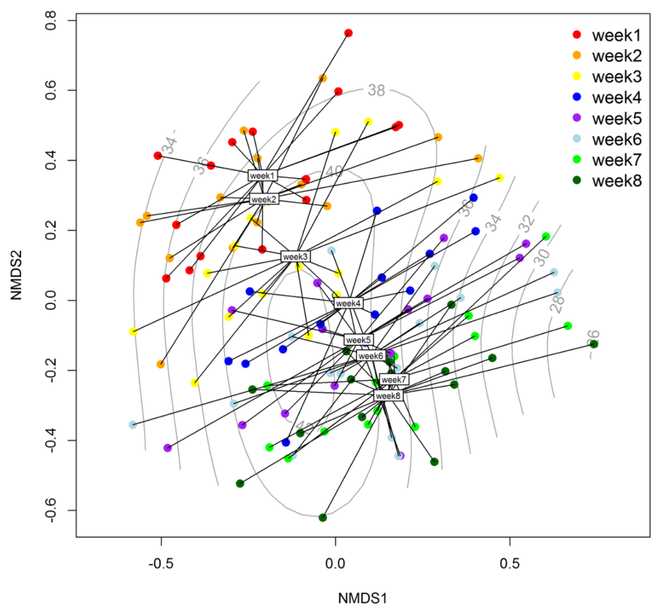

Fig. 4. NMDS ordination showing MBC samples (all arthropods) grouped by week. Surface plot shows species richness.

Acknowledgements

This work has been jointly sponsored by the Forestry Commission and the European Union (European Regional Development Fund ERDF) within the framework of the Forestry Commission Climate Change Adaptation and Biodiversity Programmes and the European INTERREG IV A 2 Mers Seas Zeeën Cross-border Cooperation Programme 2007–2013 (Project 090316016-FR MULTIFOR: Management of Multi-Functional Forests). This work also benefitted from a NERC PhD studentship grant awarded to Catherine Bruce at the University of East Anglia (2010–2013). We gratefully acknowledge the contributions of Chen-Xue Yang, Amy Eycott, Alex Robinson, Lauren Fuller and Forest Research's Technical Support Unit in the sample processing stages of the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.01.023.

References

Barsoum, N., A'Hara, S., Cottrell, J., Green, S., 2018. Using DNA barcoding and metabarcoding to detect species and improve forest biodiversity monitoring. Forestry Commission Research Note 32. Forestry Commission, Edinburgh.
Barsoum, N., Coote, L., Eycott, A.E., Fuller, L., Kiewitt, A., Davies, R.G., 2016. Diversity, functional structure and functional redundancy of woodland plant communities: how do mixed tree species plantations compare with monocultures? For. Ecol. Manage. 382, 244–256.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.
Beckschäfer, P., Mundhenk, P., Kleinn, C., Ji, Y., Yu, D.W., Harrison, R.D., 2013. Enhanced structural complexity index: an improved index for describing forest structural complexity. Open J. For. 3, 23–29.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300.
Boutin, S., Haughland, D.L., Schieck, J., Herbers, J., Bayne, E., 2009. A new approach to forest biodiversity monitoring in Canada. For. Ecol. Manage. 258, 168–175.
Brandon-Mong, G.-J., Littlefair, J., Sing, K., Lee, Y.P., Gan, H. Mi., Clare, E.L., Wilson, J., 2018. Temporal changes in arthropod activity in tropical anthropogenic forests. Bull. Entomol. Res. 1–8. https://doi.org/10.1017/S000748531800010X.
Brockerhoff, E.G., Jactel, H., Parrotta, J.A., Quine, C.P., Sayer, J., 2008. Plantation forests and biodiversity: oxymoron or opportunity? Biodivers. Conserv. 17, 925–951.
Cantarello, E., Newton, A., 2008. Identifying cost-effective indicators to assess the conservation status of forested habitats in Natura 2000 sites. For. Ecol. Manage. 256, 815–826.
Cardoso, P., Silva, I., De Oliveira, N.G., Serrano, A.R.M., 2004. Indicator taxa of spider (Araneae) diversity and their efficiency in conservation. Biol. Conserv. 120, 517–524.
Carpenter, S., McArthur, C., Selby, R., Ward, R., Nolan, D.V., Mordue Luntz, A.J., Dallas, J.F., Tripet, F., Mellor, P.S., 2008. Experimental infection studies of UK *Culicoides* species midges with bluetongue virus serotypes 8 and 9. Vet. Rec. 163, 589–592.
Chao, A., 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43 (4), 783–791.
Chirici, G., McRoberts, R.E., Winter, S., Bertini, R., Brändli, U.-B., Asensio, I.A., Barsoum, N., Bastrup-Birk, A., Rondeux, J., Marchetti, M., 2012. National Forest Inventory contributions to forest biodiversity monitoring. For. Sci. 58 (3), 257–268.
Colwell, R.K., Coddington, J.A., 1994. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. B: Biol. Sci. 345 (1311), 101–118.
Coote, L., Dietsch, A.C., Wilson, M.W., Graham, C.T., Fuller, L., Walsh, A.T., Irwin, S., Kelly, D.L., Mitchell, F.J.G., Kelly, T.C., O'Halloran, J., 2013. Testing indicators of biodiversity for plantation forests. Ecol. Ind. 32, 107–115.
Cristescu, M.E., 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of biodiversity. Trends Ecol. Evol. 29 (10), 566–571. https://doi.org/10.1016/j.tree.2014.08.001.
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. Mol. Ecol. 26 (21), 5872–5895. https://doi.org/10.1111/mec.14350.
Elbrecht, V., Vamos, E.E., Meissner, K., Aroviita, J., Leese, F., 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods Ecol. Evol. 8, 1265–1275. https://doi.org/10.1111/2041-210X.12789.
Ferris, R., Humphrey, J.W., 1999. A review of potential biodiversity indicators for application in British forests. Forestry 72, 313–328.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3, 294–299.
Foord, S., Dippenaar-Schoeman, A., Stam, E., 2013. Surrogates of spider diversity, leveraging the conservation of a poorly known group in the Savanna Biome of South Africa. Biol. Conserv. 161, 203–212. https://doi.org/10.1016/j.biocon.2013.02.011.
Forcino, F.L., Ritterbush, K.A., Stafford, E.S., 2015. Evaluating the effectiveness of the Mantel test and Procrustes randomization test for exploratory ecological similarity among paleocommunities. Palaeogeogr. Palaeoclimatol. Palaeoecol. 426, 199–208.
Forestry Commission (FC), 2017. The UK Forestry Standard: The Government's approach to sustainable forestry. Forestry Commission, Edinburgh, pp. 1–225.
Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression, second ed. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. (Accessed May 2018).
Fraser, S.M., Dytham, C., Mayhew, P.J., 2008. The effectiveness and optimal use of Malaise traps for monitoring parasitoid wasps. Insect Conserv. Divers. 1, 22–31. https://doi.org/10.1111/j.1752-4598.2007.00003.x.
Gao, T., Nielsen, A.B., Hedblom, M., 2015. Reviewing the strength of evidence of biodiversity indicators for forest ecosystems in Europe. Ecol. Ind. 57, 420–434.
Gardner, T., 2012. Monitoring Forest Biodiversity: Improving Conservation Through Ecologically-Responsible Management. Routledge, Oxford.
Gaspar, C., Gaston, K.J., Borges, P.A.V., 2010. Arthropods as surrogates of diversity at different spatial scales. Biol. Conserv. 143 (5), 1287–1294.
Geiger, M.F., Moriniere, J., Hausmann, A., Haszprunar, G., Wägele, W., Herbert, P.D.N., Rulik, B., 2016. Testing the global malaise trap program – How well does the current barcode reference library identify flying insects in Germany? Biodivers. Data J. 4, e10671.
Graves, S., Piepho, H.-P. Selzer, L., Dorai-Raj, S., 2015. multcompView: Visualizations of Paired Comparisons. R package version 0.1-7. http://CRAN.R-project.org/package=multcompView. (Accessed May 2018).
Hajibabaei, M., Baird, D.J., Fahner, N.A., Beiko, R., Golding, G.B., 2016. A new way to contemplate Darwin's tangled bank: how DNA barcodes are reconnecting biodiversity science and biomonitoring. Philos. Trans. R. Soc. London, Ser. B, Biol. Sci. 371, 20150330. https://doi.org/10.1098/rstb.2015.0330. (Accessed May 2018).
Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., de Kroon, H., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE 12 (10), e0185809.
Hartley, M.J., 2002. Rationale and methods for conserving biodiversity in plantation forests. For. Ecol. Manage. 155, 81–95.
Herrmann, S., Kahl, T., Bauhus, J., 2015. Decomposition dynamics of coarse woody debris of three important central European tree species. Forest Ecosyst. 2 (27), 1–14. https://doi.org/10.1186/s40663-015-0052-5.
Humphrey, J.W., Ferris, F., Quine, C.P., 2003. Biodiversity in Britain's Planted Forests. Forestry Commission, Edinburgh pp. i–vi + 1–118.
Humphrey, J., Bailey, S., 2012. Managing deadwood in forests and woodlands. Forestry Commission Practice Guide. Forestry Commission, Edinburgh pp. I-iv + 1-24.
Isbell, F., Gonzalez, A., Loreau, M., Cowles, J., Díaz, S., Hector, A., Mace, G.M., Wardle, D.A., O'Connor, M.I., Duffy, J.E., Turnbull, L.A., 2017. Linking the influence and dependence of people on biodiversity across scales. Nature 546 (7656), 65–72.
Ji, Y., Ashton, L., Pedley, S., Edwards, D., Tang, Y., Nakamura, A., Kitching, R., Dolman, P., Woodcock, P., Edwards, F., Larsen, T., Hsu, W., Benedick, S., Hamer, K., Wilcove, D., Bruce, C., Xiaoyang, W., Levu, T., Lott, M., Emerson, B., Yu, D., 2013. Reliable, comprehensive, and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett. 16 (10), 1245–1257.
Jukes, M., Peace, A., 2003. Invertebrate communities in plantation forests. In: Humphrey, J.W., Ferris, F., Quine, C.P. (Eds.), Biodiversity in Britain's Planted Forests. Forestry Commission, Edinburgh, pp. 75–92.

Kirkman, L.K., Smith, L.L., Quintana-Ascencio, P.F., Kaeser, M.J., Golladay, S.W., Farmer, A.L., 2012. Is species richness congruent among taxa? Surrogacy, complementarity, and environmental correlates among three disparate taxa in geographically isolated wetlands. Ecol. Ind. 18, 131–139.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2014. lmerTest: Tests for random and fixed effects for linear mixed effect models. R package version 2.0-11* – URL http://CRAN. R-project. org/package=lmerTest.

Larrieu, L., Gonin, P., 2008. L'indice de biodiversité potentielle (IBP): une méthode simple et rapide pour évaluer la biodiversité potentielle des peuplements forestiers. Revue Forestière Française 60, 727–748.

Larrieu, L., Gosselin, F., Archaux, F., Chevalier, R., Corriol, G., Dauffy-Richard, E., Deconchat, M., Gosselin, M., Ladet, S., Savoie, J.-M., Tillon, L., Bouget, C., 2018. Cost-efficiency of cross-taxon surrogates in temperate forests. Ecol. Ind. 87, 56–65.

Leather, S.R., 2018. 'Ecological Armageddon'- more evidence for the drastic decline in insect numbers. Ann. Appl. Biol. 172, 1–3.

Leese, F., Bouchez, A., Abarenkov, K., Altermatt, F., Borja, A., Bruce, K., Ekrem, T., Čiampor, F., Čiamporová-Zaťovičová, Z., Costa, F.O., Duarte, S., Elbrecht, V., Fontaneto, D., Franc, A., Geiger, M.F., Hering, D., Kahlert, M., Stroil, B.K., Weigand, A.M., 2018. Chapter Two – Why we need sustainable networks bridging countries, disciplines, cultures and generations for aquatic biomonitoring 2.0: a perspective derived from the DNAqua-Net COST Action Next Generation. In: Bohan, D.A., Dumbrell, A.J., Woodward, G., Jackson, M. (Eds.), Biomonitoring: Part 1. Elsevier Ltd., pp. 63–99.

Lenth, R., 2015. lsmeans: Least-Squares Means. R package version 2.20-23. http://CRAN.R-project.org/package=lsmeans (Accessed May 2018).

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., et al., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front. Zool. 10 (1), 34.

Lindenmayer, D.B., Likens, G.E., 2010. The science and application of ecological monitoring. Biol. Conserv. 143, 1317–1328. https://doi.org/10.1016/j.biocon.2010.02.013.

Luff, M., 2007. RES Handbook, Vol. 4, part 2: The Carabidae (Ground Beetles) of Britain and Ireland. Field Studies Council, Shropshire, UK.

MacDicken, K.G., Sola, P., Hall, J.E., Sabogal, C., Tadoum, M., De Wasseige, C., 2015. Global progress toward sustainable forest management. For. Ecol. Manage. 352, 47–56.

Mason, W.L., Connolly, T., 2014. Mixtures with spruce species can be more productive than monocultures: evidence from the Gisburn experiment in Britain. Forestry 87, 209–217.

Matthews, R.W., Matthews, J.R., 1971. The malaise trap: its utility and potential for sampling insect populations. The Great Lakes Entomologist 4 (4), 117–122.

McDonald, G.T., Lane, M.B., 2004. Converging global indicators for sustainable forest management. Forest Pol. Econ. 6, 63–70.

Morinière, J., Cancian de Araujo, B., Lam, A.W., Hausmann, A., Balke, M., Schmidt, S., Hendrich, L., Doczkal, D., Fartmann, B., Arvidsson, S., Haszprunar, G., 2016. Species identification in malaise trap samples by DNA barcoding based on NGS technologies and a scoring matrix. PLoS ONE 11 (5), e0155497. https://doi.org/10.1371/journal.pone.0155497.

National Biodiversity Network (NBN) Atlas Partnership (2017). https://nbnatlas.org/. (Accessed November 2017).

Noss, R.F., 1999. Assessing and monitoring forest biodiversity: a suggested framework and indicators. For. Ecol. Manage. 115, 135–146.

Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2016. vegan: Community Ecology Package. R package version 2.3-5. https://CRAN.R-project.org/package=vegan. (Accessed May 2018).

Paquette, A., Messier, C., 2010. The role of plantations in managing the world's forests in the Anthropocene. Front. Ecol. Environ. 8, 27–34.

Pawson, S.M., Brockerhoff, E.G., Watt, M.S., Didham, R.K., 2011. Maximising biodiversity in plantation forests: Insights from long-term changes in clearfell-sensitive beetles in a *Pinus radiata* plantation. Biol. Conserv. 144 (12), 2842–2850.

Pretzsch, H., 2017. Chapter 6: Individual tree structure and growth in mixed compared with monospecific stands. In: Pretzsch, H., Forrester, D.I., Bauhus, J. (Eds.), Mixed-Species Forests Ecology and Management. Springer-Verlag GmbH, Germany, pp. 271–336.

Puettmann, K.J., 2011. Silvicultural challenges and options in the context of global change: "simple" fixes and opportunities for new management approaches. J. Forest. 109, 321–331.

Puumalainen, J., Kennedy, P., Folving, S., 2003. Monitoring forest biodiversity: a European perspective with reference to temperate and boreal forest zone. J. Environ. Manage. 67, 5–14.

Quine, C.P., Humphrey, J.W., 2003. The future management of plantation forests for biodiversity. In: Humphrey, J.W., Ferris, F., Quine, C.P. (Eds.), Biodiversity in Britain's Planted Forests. Forestry Commission, Edinburgh, pp. 103–113.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org. (Accessed May 2018).

Roberts, M.J., 1993. The spiders of Great Britain and Ireland. Harley Books, Colchester, UK.

Sabatini, F.M., Burrascano, S., Azzella, M.M., Barbati, A., De Paulis, S., Di Santo, D., Facionia, L., Giuliarelli, D., Lombardi, F., Maggi, O., Mattioli, W., Parisi, F., Persiani, A., Ravera, S., Blasi, C., 2016. One taxon does not fit all: Herb-layer diversity and stand structural complexity are weak predictors of biodiversity in Fagus sylvatica forests. Ecol. Ind. 69, 126–137.

Shorohova, E., Kapitsa, E., 2014. Influence of the substrate and ecosystem attributes on the decomposition rates of coarse woody debris in European boreal forests. For. Ecol. Manage. 315, 173–184.

Smith, A.J.E., 2004. The Moss Flora of Britain and Ireland, 2nd ed. Cambridge University Press.

Stace, C., 2010. New Flora of the British Isles. Cambridge University Press, Cambridge.

Taboada, A., Tárrega, R., Calvo, L., Marcos, E., Marcos, J.A., Salgado, J.M., 2010. Plant and carabid beetle species diversity in relation to forest type and structural heterogeneity. Eur. J. Forest Res. 129, 31–45.

Tews, J., Brose, U., Grimm, V., Tielborger, K., Wichmann, M.C., Schwager, M., Jeltsch, F., 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. J. Biogeogr. 31, 79–92.

UK NFI, 2016. National Forest Inventory of Great Britain Survey Manual. https://www.forestry.gov.uk/fr/infd-9m8f6p. (Accessed May 2018).

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Scherer-Lorenzen, M., 2015. TreeDivNet: contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45, 29–41. https://doi.org/10.1007/s13280-015-0685-1.

Wang, Y., Naumann, U., Wright, S., Warton, D.I., 2012. mvabund: an R package for model-based analysis of multivariate data. Methods Ecol. Evol. 3, 471–474 R package version 3.6.11.

Warton, D.I., Wright, S.T., Wang, Y., 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol. Evol. 3, 89–101.

Yu, D.W., Ji, Y., Emerson, B.C., Wang, X., Ye, C., Yang, C., Ding, Z., 2012. Biodiversity soup: metabarcoding of arthropods for rapid assessment and biomonitoring. Methods Ecol. Evol. 3 (4), 613–623.

Zenner, E.K., Hibbs, D.E., 2000. A new method for modelling the heterogeneity of forest structure. For. Ecol. Manage. 129, 75–87.