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

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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.

Key words: DNA metabarcoding; malaise traps; surrogate measures of biodiversity; biodiversity indicators; forest management ; tree identity

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 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

89 inevitable high related costs and 4) difficulties in identifying species due to poor quality samples,
90 or the presence of juvenile life stages. Thus, biodiversity monitoring has tended to focus on a
91 restricted number of species that are considered to be at risk of extinction, or species that are
92 relatively easy to sample and that are taxonomically unambiguous and therefore easy to identify.

93 Alternatively, biodiversity monitoring commonly applies surrogate measures of biodiversity, or
94 'biodiversity indicators' that convey information about the wider state of the biological
95 community and which can be assessed relatively quickly and cheaply by non-experts (Ferris and
96 Humphrey, 1999; Noss, 1999; Coote et al., 2013). There are two categories of commonly used
97 surrogates: taxa-based surrogates (compositional indicators) and habitat-based surrogates
98 (structural indicators). Taxa-based surrogates refer to key taxa that are considered
99 representative of a broader segment of biodiversity (i.e. biodiversity patterns observed for the
100 surrogate taxon are generalizable to one or more taxa) (Sabatini et al., 2016). For example,
101 carabid beetles (Coleoptera: Carabidae), hoverflies (Diptera: Syrphidae), spiders (Araneae),
102 vascular plants and bryophytes are commonly cited as being potentially informative indicators of
103 the species richness of other taxa in forest settings (Ferris and Humphrey, 1999; Cardoso et al.,
104 2004; Pawson et al., 2011; Foord et al., 2013; Gao et al., 2015).

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

114 The widespread use of surrogate measures of biodiversity is, nevertheless, revealing some
115 important limitations of these methods for forest biodiversity assessments and monitoring.
116 Gaspar et al. (2010) cautioned that surrogate measures of biodiversity may show different
117 strengths of correlation depending on the geographic scale of inquiry. A recent review has
118 similarly revealed only limited evidence of the universal applicability of many commonly used
119 surrogate measures of biodiversity in different forest ecosystems (Gao et al., 2015). This is
120 because many have not been tested widely across different forest types and in different
121 bioclimatic zones (Cantarello and Newton, 2008). For certain surrogate measures of biodiversity
122 such as volume of deadwood, attempts have been made to set evidence-based threshold levels for

123 biodiversity gains (Humphrey and Bailey, 2012), although there is the complication that these
124 thresholds may need to be adjusted according to regional levels of soil fertility, the bioclimatic
125 zone, or depending on tree species present (Larrieu and Gonin, 2008). Furthermore, to reduce the
126 chances of making incorrect management decisions based on weak or ineffective surrogates that
127 may be biased in favour of a single taxon, several authors now recommend conducting
128 assessments of multiple taxonomic groups, particularly where taxonomic responses to a given
129 environmental variable (e.g. canopy cover) are unknown (Sabatini et al., 2015; Larrieu et al.,
130 2018). While this comprises a considerable sampling and sample identification effort, recent
131 advances in molecular ecology, and DNA metabarcoding in particular, are promising to make this
132 more achievable.

133 DNA metabarcoding is a powerful species identification method that uses 'next generation
134 sequencing' (NGS) technology to scale up the traditional DNA barcoding process. This allows the
135 rapid characterisation of complex samples of multiple species by sequencing their DNA barcodes
136 in parallel, simultaneously reducing the need for taxonomic expertise and the time and cost
137 required to obtain high quality biodiversity data, across a wide range of taxonomic groups, at
138 large spatial and temporal scales (Yu et al., 2012; Barsoum et al., 2018). Previous studies have
139 shown that metabarcoding arthropods generates accurate and reliable alpha and beta
140 biodiversity information at a fraction of the time and cost of traditional survey methods (Yu et al.,
141 2012; Ji et al., 2013; Morinière et al., 2016).

142 Here, we explore the potential to apply a metabarcoding approach to measure biodiversity
143 response to subtle differences in forest environmental conditions and we compare this approach
144 with the use of taxa- and habitat-based surrogate measures of biodiversity. Specifically, we
145 investigate the scope for a metabarcoding approach to provide data that can be used to: (1) detect
146 any fine-scale spatial and temporal variation in arthropod community composition in response to
147 tree species composition in plantation forest stands, (2) evaluate the biodiversity effects of
148 different forest management strategies; i.e. plantation monocultures compared with polycultures
149 and (3) identify which species or species groups of arthropods captured in malaise traps are most
150 sensitive to the composition of forest stands. We use a sampling method that is effective at
151 trapping insects from the orders Diptera and Hymenoptera (Matthews and Matthews, 1971;
152 Geiger et al., 2016; Morinière et al., 2016). Despite being among the most species rich groups of
153 arthropods, Diptera and Hymenoptera are almost always overlooked in biodiversity studies
154 because of the difficulty associated with sorting and identifying the inevitably large number of
155 specimens which tend to be characterised by small body size (Jukes and Pearce, 2003; Fraser et
156 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?

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°30' N, 0°51' E; 10-40m 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 1.5ha, 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.

187 *2.2 Data collection*

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

196 *2.2.1 Diverse arthropod taxa - Metabarcode (MBC) dataset*

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

204 *2.2.2 Taxa-based surrogate measures of biodiversity - Standard (STD) datasets*

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

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

217 *2.2.3 Habitat-based surrogate measures of biodiversity*

218 In February 2013, fourteen of the fifteen stands were surveyed to derive 16 habitat-based
219 surrogate measures of biodiversity listed in Table 2 and described in Methods A3 (Supp. Mat.);

220 stand P2 could not be surveyed because it had been harvested. Definitions and assessments of
221 stem density, deadwood and tree stumps were broadly based on those used in the UK National
222 Forest Inventory (UK NFI, 2016).

223

224 *2.3 Metabarcode protocols and data preparation*

225

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

229 A total of 1123 molecular OTUs were generated, each OTU representing a distinct species. While
230 duplicates of many of these 1123 OTUs occurred, species abundance cannot be reliably inferred
231 from multiple identical OTUs. Quality control filtering included: 1) setting a threshold of >97%
232 similarity match of OTU sequences, 2) the removal of single-read OTUs and 3) the removal of non-
233 arthropods and any species with no prior record of occurrence in the UK. This reduced the
234 number of OTUs down to 521. Of these, 67% were identifiable to species level, 8% to Genus and
235 the remaining 25% to Order level.

236 Two primary metabarcode dataframes were created from the 521 OTUs that were generated
237 from the malaise trap samples. These dataframes included a 'binary' dataframe and a 'pooled'
238 dataframe. For the binary data frame, every OTU was scored for presence-absence in each of the
239 120 malaise trap samples. This dataframe was used for: 1) visualising compositional differences
240 among samples grouped by stand type and by sample collection week (1-8) (beta diversity) and
241 2) for analysis of arthropod species richness between stand types (alpha diversity). In order to
242 increase the confidence of species occurrence, single occurrence OTUs across the 120 malaise
243 trap samples were removed from the binary dataframe.

244 For the pooled dataframe, where OTUs occurred in a single replicate stand, these were removed
245 (i.e. even if an OTU was present across all eight weeks, it was excluded if it was present in only a
246 single replicate stand). The pooled dataframe comprised species by stand data, in which the eight
247 weekly samples were pooled within each stand. For each stand, every OTU was assigned a value
248 between 0 and 8, representing the number of weeks in which it was detected. This index is not a
249 direct measure of OTU abundance, but it is expected to represent each species' contribution, over
250 time, to a forest stand's arthropod diversity. This dataset was used: (1) for comparisons with the
251 STD datasets to check for consistency of between stand type trends in species richness and (2) to
252 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 analysis: 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., 2016) 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 x 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

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

326

327 3. Results

328 3.1 Comparing species richness and community composition between stand types - MBC and STD 329 datasets

330

331 3.1.1 Taxonomic composition of MBC and STD datasets

332 MBC dataset

333 The 521 OTU's making up the MBC dataset were distributed across four arthropod Classes:
334 Arachnida, Diplopoda, Insecta and Malacostraca. Diptera were a dominant order (65% of all
335 OTUs), followed by Coleoptera (8%), Araneae, Hemiptera, Hymenoptera (each making up 6% of
336 all OTUs) and Lepidoptera (3%) (Table 3 and Supp. Mat. Table A1). Identification of OTU's to
337 species level was lowest among the Hymenoptera (52%) and Diptera (60%) and highest among
338 better known orders such as Lepidoptera (95%), Araneae (83%) and Coleoptera (90%) which
339 have comparatively high numbers of national recordings (NBN Atlas, 2017). Across all stands, a
340 total of 30 spider species were identified from 10 families. Two families of spider were unique to
341 the MBC dataset; these were orb weaver spiders (Araneidae) and mesh web weaver spiders
342 (Dictynidae) that weave webs in vegetation. A single carabid beetle species was identified in the
343 MBC dataset (*Cychrus* sp.). A number of species identified are nationally scarce or are species of
344 declining numbers (e.g. the crab spider, *Xysticus lanio*; the Green-brindled Crescent moth,
345 *Allophyes oxyacanthae*) and some (n = 46) from the Diptera, Hemiptera and Hymenoptera families
346 have never previously been recorded in the Norfolk region (highlighted in Supp. Mat. Table A1).
347 For a number of taxonomic groups (e.g. some fly and gnat families such as the Phoridae, Sciaridae,
348 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*.

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

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 stands types had significantly higher estimated species richness than Scots pine monocultures (Figure 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 lower species richness than those of the other stand types (Supp. Mat. Fig. A1).

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.

381

382 *3.1.3 Community composition between stand types*

383 An NMDS ordination of the MBC dataset showing arthropod samples grouped by stand type,
384 revealed a greater similarity in the species compositions of oak monocultures and Scots pine-oak
385 mixtures compared with Scots pine monocultures (Supp. Mat. Fig. A2). Multivariate likelihood
386 ratio (LR) tests showed significant differences in species composition across the three stand
387 types, with 30 OTUs associated with Scots pine-oak mixtures, 46 OTU's associated with oak
388 monocultures and 40 OTU's associated with pine monocultures. These included species from a
389 wide range of taxonomic Orders, although the majority were Diptera (Supp. Mat. Tables A1 and
390 A7). Conifer-associated species included one potential disease vector: the biting midge *Culicoides*
391 *scoticus*, which could be an important vector of Bluetongue virus, a serious pathogen of ruminants
392 (Carpenter et al., 2008). The mvabund analysis showed significant differences across the three
393 stand types for the majority of the MBC and STD data sets; pairwise comparisons of stand type
394 are shown in Table 4. Although some of the datasets were not significant at a 0.05 level (likely due
395 to the small sample size), there was a general trend for significant differences to be
396 predominantly driven by pine monocultures compared with the other two stand types. The
397 consistency across MBC and STD data sets provides evidence of consistent results across MBC
398 and STD measures of biodiversity.

399

400 *3.1.4 Direct comparison of MBC and STD datasets*

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

413

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), $Dev_{(1,13)} = 1{,}480$, $p = 0.02$). 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. Figure 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) (Figure 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 (Figure 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

445 The MBC and STD datasets both showed a distinctiveness in the composition of communities
446 sampled in Scots pine monocultures compared with oak monocultures for all taxonomic groups
447 assessed. In Scots pine-oak mixed stands, MBC and STD datasets also showed the same tendency
448 for communities to occupy an "intermediate" position in ordinations, with communities partially
449 comprised of component species present in either Scots pine or oak monocultures. These results
450 are in line with a growing number of studies demonstrating the effectiveness of DNA
451 metabarcoding as a method of collecting reliable biodiversity information that can be used to
452 inform management practice and policy (Ji et al., 2013; Deiner et al., 2017; Elbrecht et al., 2017).
453 In this study, the data provides evidence backing current UK forestry policy that advocates a
454 diversification in the composition of forest stands and woodlands for biodiversity gains (FC,
455 2017). Thetford Forest is dominated by pine and these results suggest that the inclusion of oak
456 stands as part of the wider mosaic of woodland stands would improve overall levels of alpha and
457 beta diversity. A notable result is the limited ordination space occupied by Scots pine-oak
458 mixtures compared with oak and Scots pine monocultures combined, with mixed stands
459 particularly failing to cover the space occupied by pine monocultures (Figure 3). This suggests
460 that in oak and Scots pine plantations, improved regional species diversity (for the taxonomic
461 groups considered here) can be achieved by creating a mosaic of pure-oak and pure-pine crops
462 rather than planting intimate mixtures of Scots pine and oak; this is because Scots pine-oak
463 mixtures would incur the loss of pine specialists.

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

477

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, Morinère 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 Morinère 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

513 (Folmer et al., 1994). This may go some way to explain the surprisingly low proportions of
514 Hymenoptera detected in our study and another malaise trap study that also used Folmer's
515 primers (Yu et al., 2012).

516

517 *4.2. Most habitat-based surrogate measures of biodiversity tested did not predict significant*
518 *differences in species assemblages between stands*

519 While some difference in structural complexity and deadwood volume were expected between
520 the different stand types based on the differing characteristics of the tree species (Mason and
521 Connolly, 2014; Shorohova and Kapitsa, 2014; Herrmann et al., 2015, Pretzsch, 2017), these
522 differences were not captured by the variables measured in this study. The range of UK-NFI
523 habitat-based surrogate measures of biodiversity that were assessed revealed a consistency in
524 the measured habitat conditions across the different stands and stand types. Stem density, stand
525 structural complexity, levels of deadwood and the number of canopy and sub-canopy tree species
526 were comparable across the stands and thus, were not useful predictors of significant species and
527 compositional differences observed in the MBC and STD datasets between the different stand
528 types. Only one variable was found to reflect the compositional differences in arthropod
529 communities found in the different stand types based on the MBC dataset; that was the
530 percentage of conifer (i.e. Scots pine) as a proportion of all trees present in the stand. These
531 results suggest that a reliance on the habitat-based surrogate measures of biodiversity applied
532 here would have led to incorrect assumptions being made about underlying patterns of
533 biodiversity (e.g. significant differences in patterns of species richness between the different
534 forest stand types might have been overlooked).

535

536 *4.3. Metabarcoding captures fine-scale temporal variations in the composition of arthropod*
537 *communities*

538 Arthropod sampling can very quickly generate extremely large, unwieldy numbers of specimens,
539 particularly less targeted sampling techniques such as malaise traps. This greatly restricts the
540 number of taxa and repeat samples than can be processed where species identification is based
541 on morphology alone (Humphrey et al., 2003; Morinière et al., 2016). Identification of species
542 using the metabarcoding approach made it possible for a high intensity and frequency of
543 arthropod assemblages to be processed. This provided insight into the very rapid changes in
544 composition of arthropod communities over an eight week period within each stand. Our results
545 showed similar rates of species assemblage change across stands and clear species associations

546 with different sampling periods indicating evident compositional shifts through time. These
547 findings underline the importance of controlling for temporal effects in sampling using malaise
548 traps, and particularly for certain taxonomic groups such as parasitoid wasps; the species
549 composition of samples collected just a couple of weeks apart can differ greatly (Fraser et al.,
550 2008; Geiger et al.,2016). Our findings additionally highlight the potential to relate finely-grained
551 temporal shifts in arthropod communities to fluctuating environmental variables in order to
552 explain the root causes of important shifts in the composition of arthropod communities. This is
553 particularly relevant when considering significant reported global declines in the abundance of
554 certain insect groups, including moths, butterflies, bees, spiders and carabid beetles (Hallmann et
555 al., 2017; Leather, 2018). The causal agents of many of these declines are not yet clear, although
556 environmental variables with a negative influence could include levels of air pollution and
557 pesticide use associated with land use intensification, and/or important variations in the
558 seasonality and range of ambient temperatures associated with global warming (Brandon-Mong
559 et al., 2018).

560

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562

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572

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Appendix A: Supplementary material

Additional Supplementary material describing Methods associated with this article are listed below.

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Methods A2: Taxa-based surrogate measures of biodiversity - Standard (STD) datasets (2.2.2)

Methods A3: Habitat-based surrogate measures of biodiversity (2.2.3)

Methods A4: Sample preparation, DNA extraction, PCR and sequencing (2.3.1)

Methods A5: Bioinformatic extraction of operational taxonomic units (OTU's) from raw sequence data (2.3.2)

Methods A6: Community composition between stand types (2.4.1.2)

Methods A7: Temporal variations in community composition – MBC dataset (2.4.3)

Additional Supplementary figures associated with this article are listed below.

Figure A1: Species accumulation curves for mixtures of Scots pine and oak (red), oak monocultures (green) and pine monocultures (blue), estimated using specaccum() function in vegan() package in R. Method = "exact" (finds the expected (mean) species richness), permutations = 9999.

Figure A2: NMDS ordination showing MBC arthropod samples grouped by stand type. Surface plot shows species richness.

Figure A3: Boxplot of second NMDS dimension by week indicating a non-linear response (flattening from ~week 5 onwards).

Additional Supplementary tables associated with this article are listed below.

Table A1 : List of species/ OTUs in the MBC dataset. Occurrence is indicated by stand type. Also indicated are species/OTUs with significant tree species associations. Species not previously recorded in the region of study (Norfolk) are highlighted with and asterisk.

Table A2 : List of spider species present in each stand type. All pitfall trap data for each given stand type combined.

Table A3 : List of carabid species present in each stand type. All pitfall trap data for each given stand type combined.

Table A4 : List of vascular plant species present in each stand type. All 2m x 2m quadrat data for each given stand type combined.

Table A5 : List of bryophyte species present in each stand type. All 2m x 2m quadrat data for each given stand type combined.

Table A6: Mean total species richness (TSR) and mean species richness (S) of Araneae, Carabidae, vascular plants and bryophytes (STD datasets) in Scots pine-oak mixed (SP/OK) and monoculture (SP, OK) stands. Standard error is given in brackets. Different lower case letters indicate a significant difference ($p < 0.05–0.001$) between stand types.

Table A7: Number of OTUs in each taxonomic Order that are significantly associated with each stand type. Based on three separate multivariate LR tests in mvabund with binomial errors, malaise.trap resampling and 999 bootstrap iterations. Each analysis tested one stand type against the other two (pooled).

Table A8: Number of OTUs in each taxonomic group that are significantly associated with the first half (weeks 1-4; August) and second half (weeks 5-8; September) of the sampling period. Based on LR tests in mvabund with binomial errors, pit.trap resampling and 999 bootstrap iterations.

*Manuscript (revision changes marked)
Click here to download Manuscript (revision changes marked): N Barsoum et al DNA metabarcoding vs surrogates_MANUSCRIP
Click here to view linked References

Figure 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.

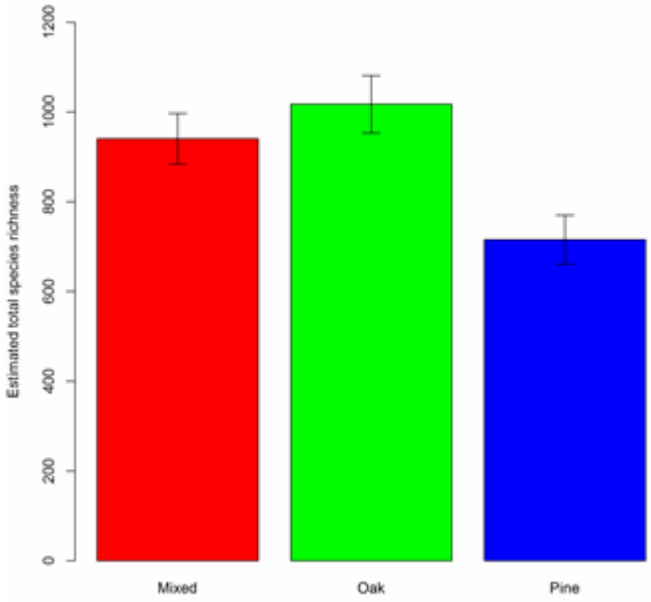
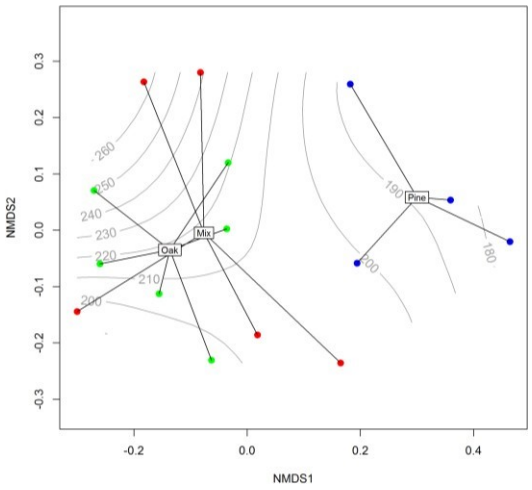
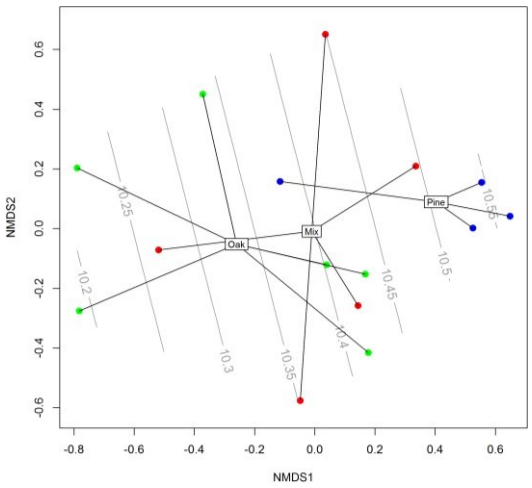


Figure 2. Non-metric multidimensional scaling (NMDS) ordinations (A-F) of MBC datasets (all arthropods, Araneae only) and STD datasets (spiders, carabids, vascular plants, bryophytes) showing samples grouped by stand type. Surface plot shows species richness.

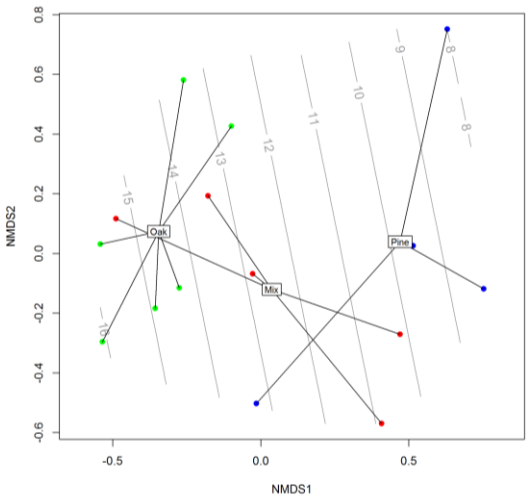
A - MBC, All arthropods, malaise traps



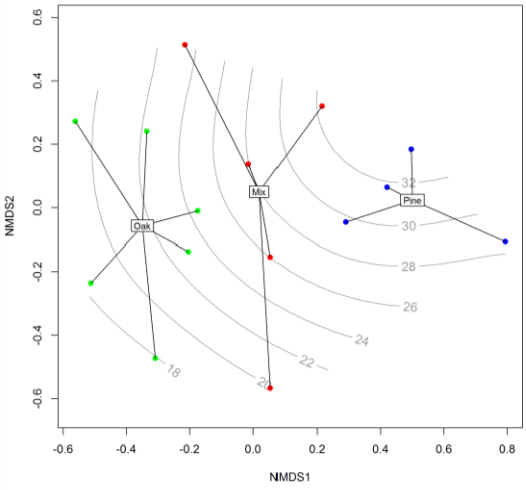
B - MBC, Araneae, malaise traps



C - STD, Carabidae, pitfall traps

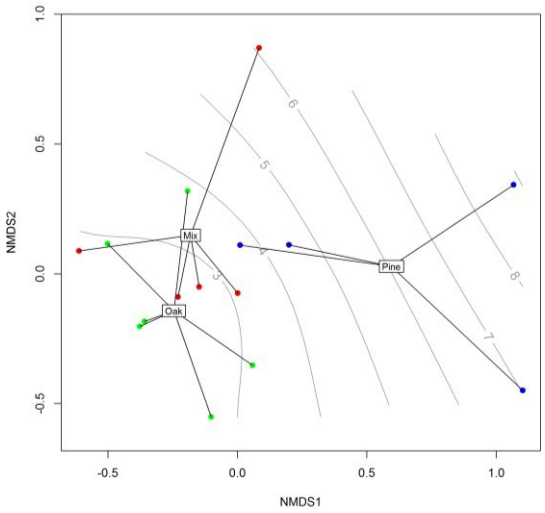


D - STD, Araneae, pitfall traps



E - STD, bryophytes, quadrats

F - STD, vascular plants, quadrats


NMDS2
NMDS1
Mix
Oak
Pine
6
5
4
3
8
7
1.0
0.5
0.0
-0.5
-0.5
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0.5
1.0

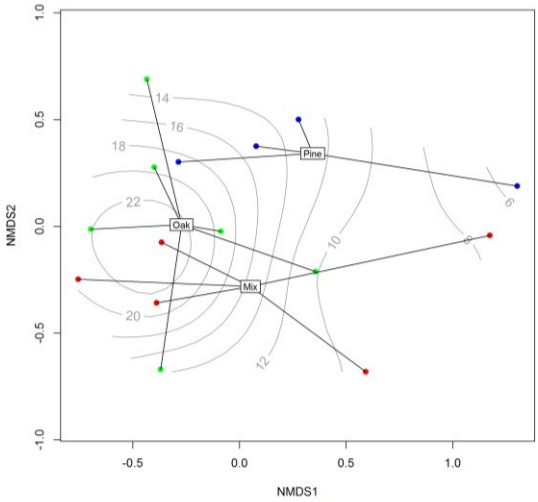
NMDS2
NMDS1
Pine
Oak
Mix
14
16
18
22
20
12
10
8
6
1.0
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0

Figure 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.

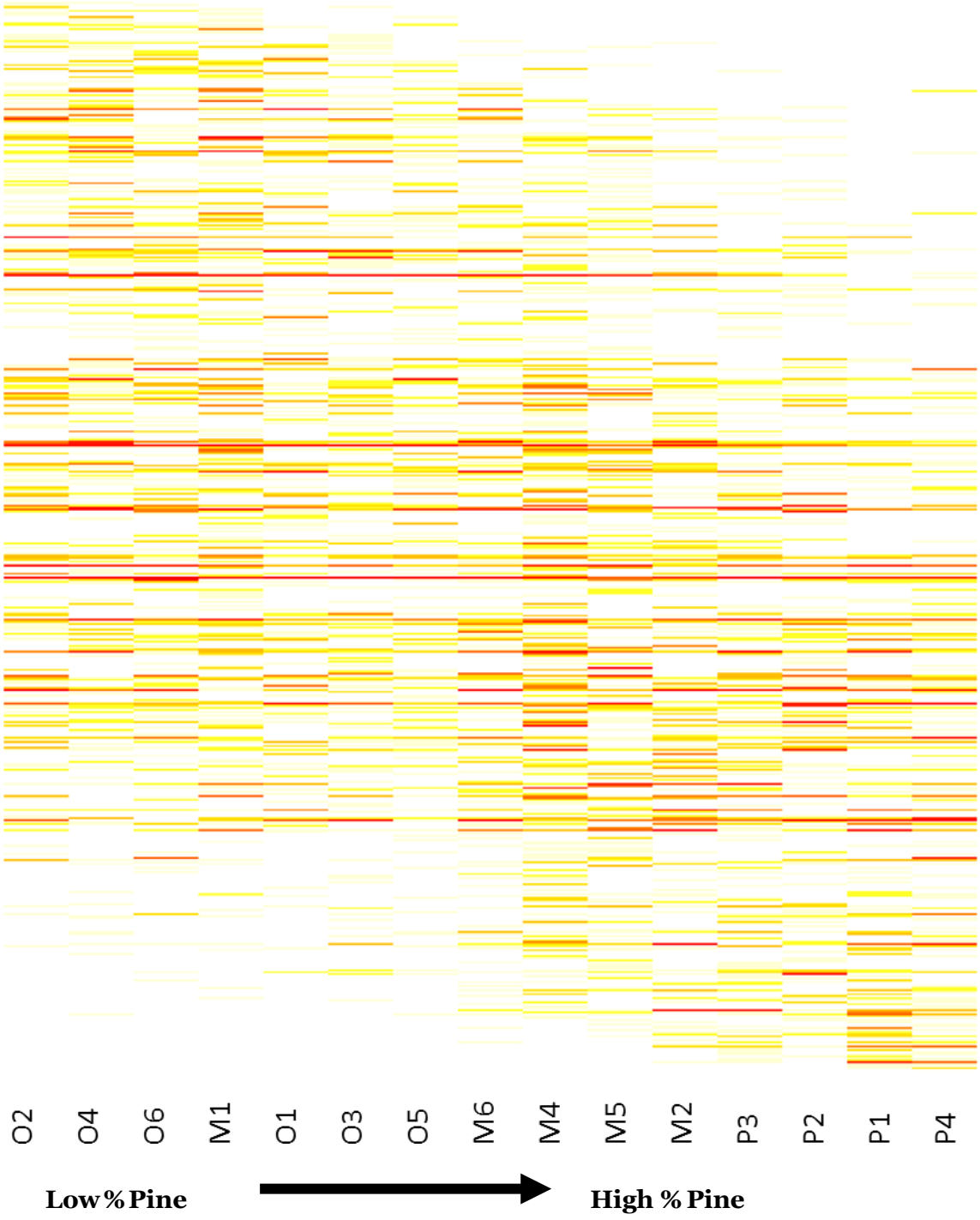

Figure 4. NMDS ordination showing MBC samples (all arthropods) grouped by week. Surface plot shows species richness.

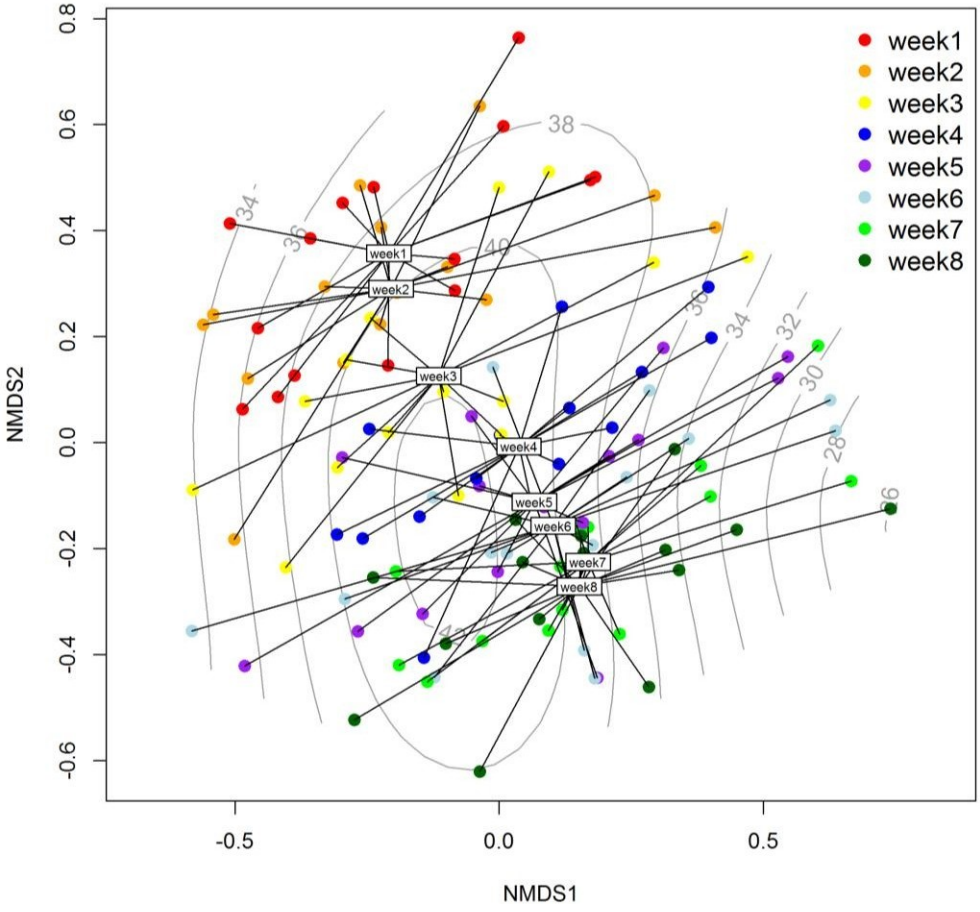

Table 1. Summary characteristics of 15 study stands in the Thetford Forest region.

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.

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 900m^2 block
Crop density	Number of crop stems (i.e. Scots pine and/or oak) in 900m^2 block
Non-crop density	Number of non-crop stems in 900m^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

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

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

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**