Title

Relative contribution of ancient woodland indicator and non-indicator species to herb layer distinctiveness in ancient semi-natural, ancient replanted, and recent woodland.

Running title

AWI and non-AWI species in ancient and recent woodland

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1. Abstract and keywords

Questions: The floristic distinctiveness of ancient woodland relative to recent woodland is commonly measured by Ancient Woodland Indicator (AWI) species richness. However, focusing on a pre-defined subset of species means that wider community-level differences may be overlooked. Can ancient semi-natural, ancient replanted, and recent woodland herb layer communities be distinguished by alpha, beta, and gamma diversity? How are any differences partitioned across AWI and non-AWI species communities?

Location: Cotswolds, South-West UK.

Methods: To quantify AWI and non-AWI responses to stand history in ancient semi-natural, ancient replanted, and recent woodland, we conducted floristic surveys of 45 sites. Using a modelling approach, we tested the relative and additive contribution of alpha scale AWI and non-AWI species richness to woodland distinctiveness. Ordination was applied to analyse beta species composition distinctiveness, and multilevel pattern analysis was used to examine which species were significant contributors to gamma scale richness differences.

Results: AWI species richness models significantly distinguished ancient semi-natural woodland from both ancient replanted and recent woodland at the alpha scale. For the classification of ancient semi-natural woodland and recent woodland, the hierarchical inclusion of non-AWI alpha richness resulted in a superior and more significant model. AWI gamma richness was numerically similar for all three woodland categories, whereas non-AWI was more varied. AWI and non-AWI species composition showed significant beta diversity differences among all woodland types, with six species being significant drivers of differences.

Conclusions: Our results have revealed previously undetected complexity in the contributions of AWI and non-AWI species to floristic distinctiveness of ancient woodland. In addition to traditional AWI species, the non-AWI assemblage also exhibited a sensitivity to habitat continuity that: (a) adds weight to the argument that ancient woodland is floristically distinct from recent woodland; and (b) provides a useful measure of success for ancient replanted woodland restoration.

Keywords: Ancient woodland species, richness, diversity, alpha, beta, gamma.

Introduction

The floristic distinctiveness of ancient woodland is a well-established concept and provides strong justification for ancient woodland being a conservation priority (Glaves, Rotherham, Wright, Handley, & Birkbeck, 2009; Goldberg, Kirby, Hall, & Latham, 2007; Rotherham, 2011). In England, ancient woodland is defined as land that has been continuously wooded since at least 1600 (Goldberg et al., 2007). This includes both Ancient Semi-Natural Woodland (ASNW) and Plantations on Ancient Woodland Sites (PAWS), the latter being areas of ASNW that were felled and immediately replanted for timber production in the 20th century with no intervening agricultural land-use. Consequently, PAWS retain important features of the original semi-natural habitat such as ground, herb and shrub layer communities, and unploughed, unimproved soil (Pryor, Curtis & Peterken, 2002). Recent woodland is that established since 1600. Although terminology can vary, the concept of ancient, continuous or old-growth woodland is recognised internationally, including mainland Europe (e.g. Sabatini et al., 2018), North America (e.g. McMullin & Wiersma, 2019), South America (e.g. Barlow et al., 2007), Asia (e.g. Miura, Manabe, Nishimura, & Yamamoto, 2002), Africa (e.g. Lawton et al., 1998), and Australasia (e.g. Rudel et al., 2005).

Accurate identification of ancient woodland is necessary due to the habitat’s prominence in legislative frameworks. In the UK, forestry policy places strong emphasis on the protection and conservation of ancient woodland not only in terms of maintaining the existing area of woodland but also for PAWS restoration to meet Aichi Target 15 (DEFRA, 2013; HM Government, 2018; SCBD, 2012). Moreover, both ASNW and PAWS are accorded high (and equal) protection in the National Planning Policy Framework due primarily to their distinctive ecology (Ministry of Housing, Communities and Local Government, 2019).

Previous research has shown a strong affinity between some woodland species, such as Herb Paris (*Paris quadrifolia*) and English Bluebell (*Hyacinthoides non-scripta*), and long-established, continuously wooded habitat (e.g. Atkinson, Bailey, Vaughan, & Memmott, 2015; Kelemen, Kriván, & Standovár, 2014; Schmidt et al., 2014). Such species are often regarded as Ancient Woodland Indicator (AWI) species: vascular plants that are particularly, but not necessarily exclusively, associated with ancient woodland (Glaves et al., 2009). The association is explained by their preference for temporally-stable environments with limited environmental and anthropogenic disturbance, together with poor dispersal and/or colonisation ability (Kimberley, Blackburn, Whyatt, Kirby, & Smart, 2013). The presence of AWI species thus contributes to the evidence used to designate a woodland as ancient (Glaves et al., 2009) and may be used as an indicator of PAWS restoration success.

In contrast to the importance placed on AWI species, generalist species (i.e. the non-AWI species) are rarely explicitly used in assessment of woodland age. Non-AWI species are usually subsumed in a simple count of all vascular herb layer species, despite there being little consensus in the literature in whether non-AWI species assemblages differ significantly between ASNW/PAWS and recent woodland (Baeten, Hermy, van Daele, & Verheyen 2010; Kelemen et al., 2014; Sciama, Augusto, Dupouey, Gonzalez, & Domínguez, 2009). Moreover, despite the restoration potential of coniferous PAWS leading to an increasing focus on ASNW-PAWS comparison research in the UK (e.g. Atkinson et al., 2015; Bergès et al., 2017; Brown, Curtis, & Adams, 2015), very few studies (e.g Brunet et al., 2011) have explicitly examined this for predominantly broadleaf PAWS. In addition, the use of negative or reverse indicators (i.e. recent woodland indicator species that are associated primarily with recent woodland that might, through their absence, be indicative of ancientness) is gaining currency as a tool to distinguish ASNW and PAWS from recent woodland (Glaves et al. 2009; Kelemen *et al*., 2014; Kirby & Morecroft, 2011; Webb & Goodenough, 2018). It is therefore important to consider a broader approach to floristic assessment.

When measured at the alpha (site-specific) scale, studies typically show that ASNW is significantly richer in herb layer AWI (or woodland specialist) species compared to recent woodland (Brunet et al., 2011; Kelemen et al., 2014; Hofmeister, Hošek, Brabec, Hédl, & Modrý, 2013; Orczewska, 2009). As such, ancient woodlands are considered important refugia for the conservation of specialist and protected plant species, and act as potential source populations for restored or recent woodland (Brunet et al., 2011; Jacquemyn, Butaye, & Hermy, 2003; Petit et al., 2004). However, alpha richness does not account for species identity, rather it is a simple count of the number of species accorded AWI status. As such, alpha richness alone does not allow understanding the distinctiveness of floristic communities. Moreover, the mean alpha richness of an 'average' ASNW or PAWS does not represent any particular woodland, which reduces the usefulness of this approach in informing management of specific sites.

In response to the limitations of alpha diversity in an applied context, a multi-scale approach to landscape ecology is increasingly implemented (e.g. Iknayan, Tingley, Furnas, & Beissenger, 2014; Thornton, Branch, & Sunquist, 2011). The combined study of alpha, beta, and gamma diversity is particularly valued in terms of informing management of anthropogenically fragmented habitats (Thornton et al., 2011). Gamma diversity is valued as part of this multi-scale approach (Lososová et al., 2011), although seemingly never within an ancient woodland context. Increasingly, ecological studies employ species-based beta indices or ordination techniques to compare communities to inform ecological restoration or connectivity planning (Anderson et al., 2011; Socolar, Gilroy, Kunin, & Edwards, 2016). This use of beta diversity measures has been shown to be highly effective in detecting significant community differences between the herb layers of ancient woodland and recent broadleaf woodland, ancient coniferous plantations, or woodlands with different restoration treatments (Atkinson et al., 2015; Bergès et al., 2017; Coote, French, Moore, Mitchell, & Kelly, 2012; Jamoneau, Chabrerie, Closset-Klopp, & Decocq, 2012).

This study examines both AWI and non-AWI species communities of ASNW, PAWS, and recent woodlands using alpha, beta, and gamma metrics. Despite the increasing policy focus on ancient woodland meaning that enhanced understanding of community processes at a range of scales is vital, this is seemingly the first time that all three (alpha, beta, and gamma) diversity measures have been considered together for the same sites in the context of comparing ancient and recent woodland. We aim to quantify whether there are differences in herb layer species richness and composition of ancient, ancient replanted, and recent woodlands, and, if so, to determine: (1) in what aspect of diversity (alpha, beta or gamma) those differences occur; and (2) how differences are partitioned across the AWI and non-AWI species communities. Our study will thus allow consideration of whether, by focussing on the AWI concept to identify and characterise ancient woodland, subtler community-level differences between ancient and recent woodlands have been overlooked. We discuss the implications of our findings in relation to the identification of likely ancient woodland sites and the need to deliver maximally effective conservation of valued floristic communities, especially at a landscape scale.

Methods

Study area

This study was carried out in the Cotswold Hills (South-West UK, centred on 51.93N, 1.96W, elevation 150-270m (Fig. 1). The annual mean diurnal temperature was 8.6-14.7°C and precipitation 843mm (MET office, 2019). The prevalent substrate is Jurassic oolitic limestone. This region is recognised as a priority area for ancient woodland conservation, having twice the proportion of ancient woodland cover compared to the national average: 4.6% versus 2.3% (Atkinson & Townsend, 2011; Cotswold Conservation Board, 2018).

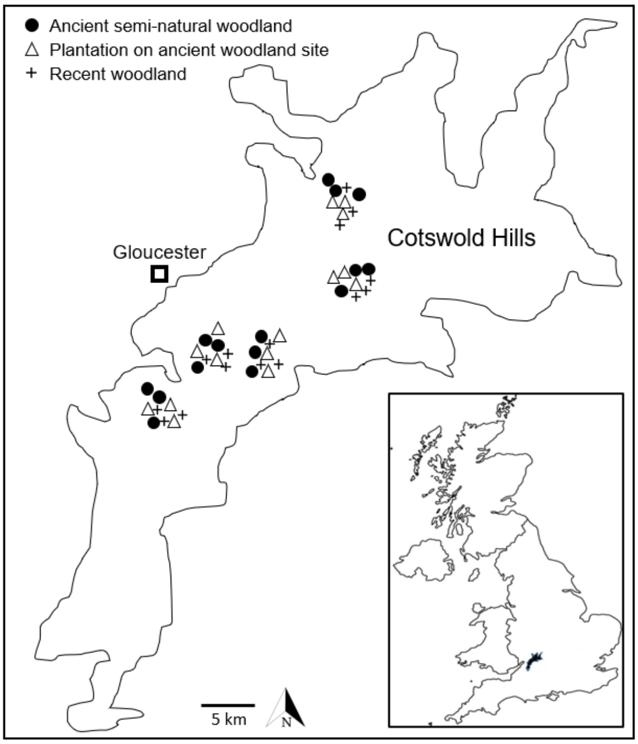

Figure 1. Study sites within Cotswold Hills. Location within UK shown on inset. There were three ancient semi-natural woodlands, three plantations on ancient woodland sites, and three recent woodlands within each of the five Strategic Nature Areas, totalling 45 sites. Sites markers are not to scale.

Site selection

Woodland sites (n=45) were sampled from five Strategic Nature Areas that are recognised as priority areas for ancient woodland with potential for increased connectivity or restoration. To mitigate any effects of spatial autocorrelation (Legendre, 1993), three ASNW, three PAWS, and three recent woodland sites were randomly selected in each of the five Strategic Nature Areas giving an overall sample size of (ASNW (n=15), PAWS (n=15), recent (n=15). The ancient status of a woodland was determined using the classifications on the definitive Multi-Agency Geographic Information for the Countryside (MAGIC) mapping tool, available at magic.defra.gov.uk. A site was delimited either as an isolated woodland patch surrounded by another land-use, or a discrete homogenous compartment within a larger woodland comprising numerous compartments of various canopy cover, management, and continuity types which therefore could not be treated as a single site.

To minimise variation in environmental variables, geological, spatial and basic floristic parameters were determined using ArcGIS and by walkover surveys prior to site sampling. All sites were located on limestone, occurred between 170-270 m.a.s.l. Woodland sites were between 0.30 and 10 hectares (e.g. Brunet et al., 2011; Kolk & Naaf, 2015). To account for site size, a Mann-Whitney U analysis was conducted: ASNW sites were significantly larger than both PAWS (p=0.04) and recent sites (p=0.04) (which were not significantly different in size (p=0.967)) (Appendix 1). All sites were National Vegetation Classification W8 woodland (*Fraxinus excelsior – Acer campestre – Mercurialis perennis*). All sites had at least 70% broadleaf canopy cover (Bergès et al., 2017; Kolk & Naaf, 2015).

Vegetation survey

Within each woodland site, herb layer vegetation was sampled in five 4x4m systematically located plots, based on an adapted National Vegetation Classification protocol (Hall, Kirby, & Whitbread, 2004). This gave 225 plots overall (45 sites split evenly between ASNW, PAWS and recent * 5 plots in each site). All vascular plant species occurring in the herb layer (excluding woody species and tree seedlings) were surveyed. Analysis was restricted to the herb layer community as this is recognised as the most sensitive indicator of past land-use (Gilliam, 2007). Regional AWI lists for South-West and South England (Rose, 1999), and neighbouring county lists (Glaves et al., 2009; Kirby, 2004) were used to classify the recorded species into AWI and non-AWI categories. Nomenclature follows Stace (2019).

To reduce the influence of edge effects (Swallow and Goodenough, 2017), plots were always located ≥15m from any edge. Internal microhabitats, such as streams and glades, were avoided (Honnay, Hermy, & Coppin, 1999). To account for the phenology of woodland species (e.g. Brunet et al., 2011), three surveys were conducted in 2014 and 2015 covering spring, summer, and autumn. This ensured that ephemeral spring species (e.g. Wood Anemone (*Anemone nemorosa*)), summer species (e.g. Enchanter’s Nightshade (*Circaea lutetiana*)), and autumn species (e.g. Autumn Crocus (*Colchicum autumnale*)) were all present within the survey window. Data were pooled at plot level to give a robust vegetation audit for each site and avoid temporal pseudoreplication.

Statistical methods

To examine patterns in AWI and non-AWI communities, diversity was described as richness data (number of species per site for alpha diversity and across all sites for gamma diversity) or presence data for individual species (beta diversity) for all analyses as per Legendre, Borcard, & Peres-Neto, (2005). Species presence was selected for the present study, because it is the presence, rather than abundance, of AWI species that contributes to the evidence for ancient woodland status (Rackham, 2008; Kirby, 2004; Rose, 1999).

Because alpha richness of AWI species is central to the identification of ancient woodland (Glaves et al., 2009), it was important to first establish the separate contribution of AWI and non-AWI species richness to ASNW, PAWS, and recent woodland herb layer distinctiveness. Both variables were approximately normally distributed. As AWI richness is used in practice as a predictor of woodland age, a predictive modelling analysis was selected. Univariate discriminant function analysis was applied to AWI richness between ASNW-PAWS, ASNW-recent, and PAWS-recent woodland. The same procedure was separately applied to non-AWI species richness. Testing between two woodland types enabled comparison with prior studies where two woodland types, commonly ASNW and recent, were compared. To test for any advantage of non-AWI inclusion in addition to AWI richness, hierarchical multivariate models were applied to the same woodland type groupings. AWI richness was entered, followed by hierarchical entry of non-AWI richness. For each multivariate model, collinearity was within accepted limits: VIF < 10 (Myers, 1990); tolerance >0.2 (Menard, 1995). Model classification accuracy was undertaken on a cross-validated dataset whereby the model was calculated repeatedly, each time leaving out a different individual case, which was then itself classified. In this way, model classification accuracy was not confounded by the model being built and tested using the same dataset (Shaw, 2009).

Gamma diversity of AWI and non-AWI species was described as the cumulated species richness across all plots of each woodland type.

To visualise beta variability among ASNW, PAWS and recent woodland, separate AWI and non-AWI presence/ absence dataframes were analysed using Non-metric Multi-Dimensional Scaling (NMDS) (Atkinson et al., 2015) using the 'metaMDS' function in the Vegan package for R (Oksanen et al., 2017). Stress was maintained <0.20 by using three dimensions (Gardener, 2014). NMDS permitted choice of the Jaccard presence/ absence distance measure (Naaf and Wulf, 2010) which was calculated between all permutations of each of the 45 woodland sites. Ordination plots were created using the 'ordiplot' function in BiodiversityR (Kindt and Kindt, 2017).

Inferential testing for beta species composition differences was conducted between ASNW-PAWS, ASNW-recent and PAWS-recent woodland via permutational multivariate analysis of variance (PERMANOVA) in the 'Adonis' (Analysis of Dissimilarity) function in Vegan for R. To establish the contribution of the mean (difference in species composition) and variance (within-group heterogeneity in composition among sites) to PERMANOVA results, variance (mean Jaccard distance to centroid) was tested using the 'betadisper' function in the Vegan package for R (Anderson, 2006). Testing for significant beta community variance between woodland continuity types was conducted using the Tukey's HSD wrapper. In this study, therefore variance was considered informative in terms of quantifying the consistence of species composition across sites of each woodland type rather than a potentially confounding effect (Warton, Wright, & Wang, 2012).

Finally, to establish which species were driving any significant differences in beta diversity among ASNW, PAWS, and recent woodland communities, Multilevel Pattern Analysis was applied to a combined dataframe of all AWI and non-AWI species (De Caceres and Jansen, 2016; Dufrene and Legendre, 1997).

Results

Alpha and gamma richness

A total of 70 herb layer species was recorded across all ASNW, PAWS, and recent woodland sites, of which 26 were classified as AWI species and 44 as non-AWI species (Appendix S1). In ASNW, PAWS, and recent woodland, mean AWI species alpha richness was 8.47, 6.07, and 5.13, and for non-AWI species 6.53, 6.33, and 8.93 respectively (Fig. 2).

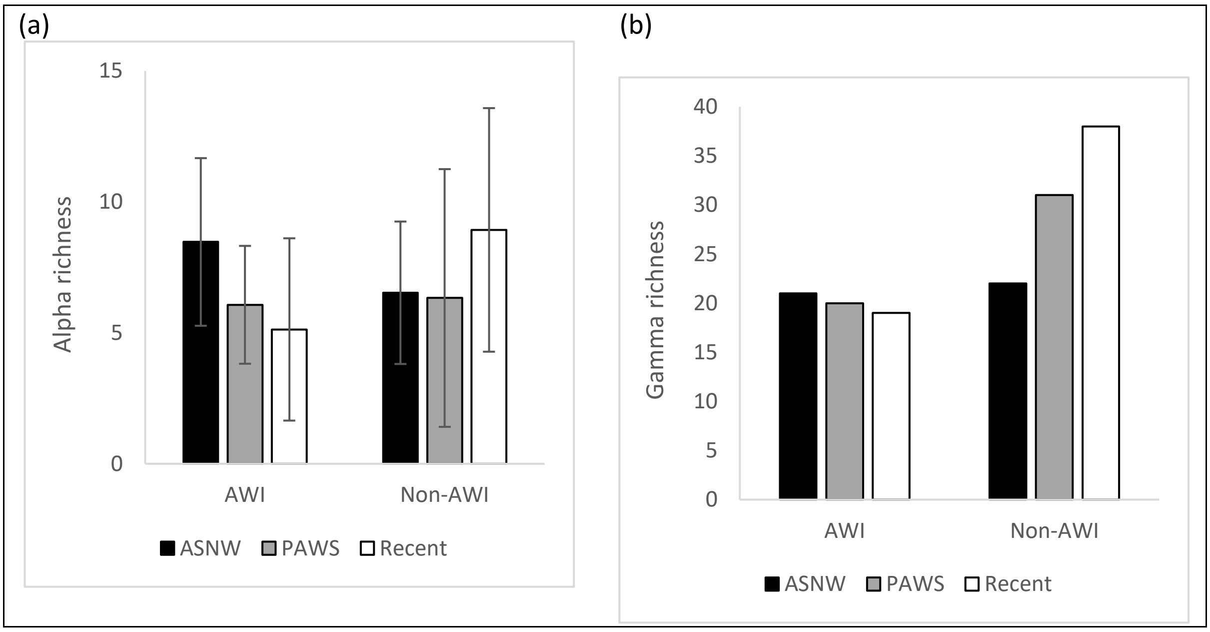

Figure 2. AWI and non-AWI species richness in Ancient Semi-Natural Woodland (ASNW) (n=15), Plantations on Ancient Woodland Sites (PAWS) (n=15), and recent woodland (n=15) for: (a) alpha scale mean richness (± s.d.) and: (b) gamma scale cumulated total from all sites of each woodland type.

Discriminant function analysis showed AWI alpha richness to significantly and strongly differentiate ASNW from both PAWS and recent woodlands (Table 1), with high AWI richness associated with ASNW in both cases. However, AWI richness showed no significant ability to classify sites as PAWS versus recent woodland. When tested independently, non-AWI richness did not significantly differentiate between any of the woodland types. For PAWS-recent comparison, non-AWI richness exhibited a stronger classification accuracy compared to AWI but was not a significant predictor of woodland type. However, for the ASNW-recent classification, the hierarchical inclusion of non-AWI richness in addition to AWI richness resulted in a superior and more significant model compared to the univariate models, with increased significance and improved classification accuracy (Table 2).

Table 1. Relative contribution of Ancient Woodland Indicator (AWI) and non-AWI richness to woodland type distinctiveness at the alpha scale. Tests between Ancient Semi-Natural Woodland (ASNW) (n=15), Plantations on Ancient Woodland Sites (PAWS) (n=15), and recent woodland (n=15). Univariate discriminant function analysis and multivariate where non-AWI richness was added hierarchically after AWI richness. Asterisks indicate: * p≤0.05; ** p≤0.01.

Woodland types compared	Models and variables	Percentage classification accuracy (50% expected *apriori*)	Wilks Lambda	Chi	d.f.	p
ASNW-PAWS	AWI	70	0.832	5.042	1	0.025*
	Non-AWI	30	0.999	0.019	1	0.891
	Hierarchical	55	0.807	5.794	2	0.055
ASNW-recent	AWI	77	0.790	6.483	1	0.011*
	Non-AWI	63	0.904	2.776	1	0.096
	Hierarchical	80	0.694	9.875	2	0.007**
PAWS-recent	AWI	57	0.974	0.737	1	0.391
	Non-AWI	70	0.927	2.089	1	0.148
	Hierarchical	60	0.887	3.234	2	0.199

At the gamma scale, all three woodland types contained numerically more non-AWI than AWI species. ASNW, PAWS, and recent woodland exhibited very similar AWI cumulated totals with 21, 20, and 19 AWI species respectively (from a total of 26) (Fig. 2). In terms of non-AWI species, there was more variation among ASNW, PAWS and recent woodland at 22, 31, and 38 respectively (from a total of 44) (Fig. 2).

Beta diversity

All three woodland types were significantly differentiated from each other by both AWI and non-AWI communities in terms of mean composition and/ variance.

For AWI species, beta diversity differed significantly between all woodland type comparisons (Fig. 3a, Table 3). ASNW and recent woodland exhibited the strongest contrast in AWI species composition, and PAWS and recent communities were significantly different. For AWI species, woodland continuity type explained between 6% and 8% of the mean difference in species composition, based on the model R^2 values (Table 3). Variance testing showed no significant differences between the woodland pairings (Table 3): the significant differences in mean species composition are therefore attributable to compositional differences rather than within-group heterogeneity.

For non-AWI species, all woodland type comparisons showed highly significant differences in beta diversity in mean composition and/ or variance. The within-group consistency of ASNW non-AWI communities is notable (Fig. 3b). ASNW and PAWS largely comprised the same species but PAWS exhibited significantly greater within-group heterogeneity than

ASNW (Fig. 3b; Table 2). For ASNW-recent, the significant mean difference cannot be entirely attributed to difference in species composition, due to a significant outcome for the variance comparison (Table 2). However, the ordination plot does exhibit a spatial distinction in terms of species present (Fig 3b), suggesting ASNW and recent non-AWI communities differ in both species present and range of plant assemblages, such that both measures of difference are important. PAWS and recent woodland have a significantly different mean species composition with no influence of within-group variance (Table 3). Woodland continuity type explained between 6 and 12% of the mean difference in species composition (Table 3).

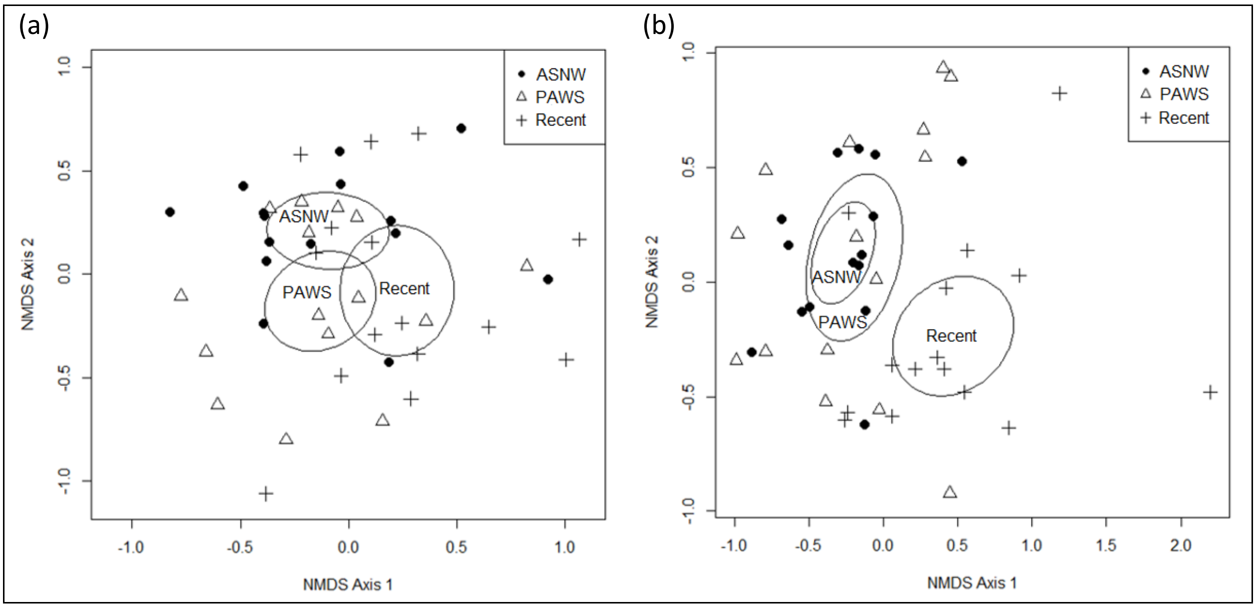

Figure 3. Beta herb layer species composition of: (a) Ancient Woodland Indicator (AWI) species and; (b) non-AWI species. Non-metric Multi-Dimensional Scaling with Jaccard distance. Ancient Semi-Natural Woodland (ASNW) (n=15); Plantations on Ancient Woodland Sites (PAWS) (n=15); recent woodland (n=15). Mean species composition is the central point within 95% confidence interval

ellipse. Ancient Woodland Indicator species (26 species) (stress = 0.167). Non-Ancient Woodland Indicators species (44 species) (stress = 0.130). See Table 2 for inferential results.

Table 2. (a) Ancient Woodland Indicator (AWI) and (b) non-AWI herb layer beta species composition differences in Ancient Semi-Natural Woodland (ASNW) (n=15), Plantations on Ancient Woodland Sites (PAWS) (n=15) and recent woodland (n=15). Cumulated number of species in each pair = n.

	(a) Ancient Woodland Indicator species			(b) Non-Ancient Woodland Indicator species		
	ASNW-PAWS	ASNW-recent	PAWS-recent	ASNW-PAWS	ASNW-recent	PAWS-recent
Species	n = 25	n = 23	n = 24	n = 32	n = 39	n = 44
Mean	p = 0.043*	p = 0.008**	p = 0.048*	p = 0.070	p = 0.001***	p = 0.002**
R^2	0.058	0.078	0.059	0.058	0.121	0.088
Variance	p = 0.316	p = 0.053	p = 0.289	p = 0.006**	p = 0.029*	p = 0.889
F value	1.044	4.087	1.168	9.026	5.325	0.020

According to Multilevel Pattern Analysis, a small number of species is significantly associated with one or two woodland continuity types (Table 3). The only significant AWI species, *Paris quadrifolia,* was associated with ASNW. *Primula veris* and *Urtica dioica* were significantly associated with recent woodland. *Asplenium scolopendrium*; *Brachypodium sylvaticum*; *Dryopteris dilatata* were all associated with PAWS alone or PAWS in conjunction with either ASNW or recent woodland.

Table 3. AWI and non-AWI species significantly affiliated with Ancient Semi-Natural Woodland (ASNW), Plantations on Ancient Woodland Sites (PAWS), and/or recent woodland based on Multilevel Pattern Analysis. Association Index 0-1. Number of occurrences across 45 woodland sites displayed in parentheses. * = AWI.

Species	Affiliated group(s)	Association Index	*p* value
Dryopteris dilatata (6)	PAWS	0.632	0.003
Urtica dioica (15)	recent	0.667	0.007
Brachypodium sylvaticum (29)	ASNW PAWS	0.814	0.012
Paris quadrifolia (9) *	ASNW	0.602	0.013
Asplenium scolopendrium (10)	PAWS recent	0.577	0.033
Primula veris (4)	recent	0.516	0.036

Discussion:

Results show that the relative contribution of AWI and non-AWI species to herb layer distinctiveness among ASNW, PAWS and recent woodland categories is complex and varies according to biodiversity metric.

Alpha and gamma diversity

In agreement with numerous studies of AWI species or woodland specialists (e.g. Hofmeister et al., 2013; Kelemen et al., 2014; Orczewska, 2009), higher alpha scale AWI species richness was significantly associated with ASNW, distinguishing it from recent woodland. ASNW is typically characterised by woodland specialist species adapted to the abiotic conditions and traditional management of ancient woodland, many of which are considered as indicator species (Glaves et al., 2009). The prevalence of these species in ASNW is attributed to a number of factors. Life traits such as late maturity, high longevity, low fecundity, and rhizomatous spread mean long-continuity habitats with minimal disturbance are required for the persistence of AWI species populations (Hermy, Honnay, Firbank, Grashof-Bokdam, & Lawesson, 1999; Kimberley et al. 2013). These traits, combined with fragmented woodland distribution, have been shown to reduce AWI dispersal to recent woodland (Brunet et al., 2011; Kimberley, Blackburn, Whyatt, & Smart, 2014), accounting for the distinction in AWI richness between ASNW and recent woodland.

Although ASNW sites were, on average, significantly larger than both PAWS and recent sites, there are conflicting findings among prior studies in terms of the influence of site size on

AWI or woodland specialist species richness. Several studies have revealed significantly more AWI or specialist species in larger sites (e.g. Jacquemyn et al., 2003; Kimberley et al., 2014; Petit et al., 2004). However, in agreement with Honnay et al. (1999), the study by Hofmeister et al. (2013), found patch size to be a weak explanatory factor of ancient woodland species. This inconsistency in findings is likely explained by a combined influence of landscape effects rather than patch size influence *per se*.

Modelling revealed that alpha scale AWI richness in predominantly broadleaf PAWS is more akin to that of recent woodland than ASNW, with a significant distinction between ASNW and PAWS but not between PAWS and recent woodland. There are seemingly no directly comparable prior studies of ASNW and broadleaf PAWS. Increased light levels, due to high canopy openness (Brown, Curtis, and Adams, 2015) and removal of the shrub layer (Kirby et al., 2014), in plantations are likely explanations for reduced richness due to absence of the most shade-adapted woodland specialists such as *Paris quadrifolia*.

The potential of PAWS to recover the flora of ASNW through restoration (Bergès et al., 2017; Harmer, Morgan, and Beauchamp, 2011; Pryor, Curtis, and Peterken, 2002) lends PAWS the same protection status as ASNW in national conservation policy and legislation (Ministry of Housing Communities and Local Government, 2019). This finding adds weight to the use of alpha AWI species richness as a measure of broadleaf PAWS restoration success.

Gamma scale AWI richness counts revealed that both PAWS and recent woodland supported a cumulated richness of AWI species comparable to ASNW. This important finding highlights the potential of PAWS and recent woodland to support AWI species, including those with protected status. Additionally, PAWS and recent woodlands have a value as source populations for AWI dispersal to restored, or newly created woodland.

Considering absolute rather than relative alpha AWI species richness among ASNW, PAWS, and recent woodland, mean richness values place all three woodland types on average into the categories of ‘very good’ or ‘good’ according to thresholds used by some organisations to assess woodland quality (Glaves et al., 2009). This is despite exclusion of woody AWI species in the present study. It is possible that recent woodland AWI species richness is elevated due to colonisation credit (Naaf and Kolk, 2015) due to all sites being located in landscapes with relatively high ancient woodland land cover. However, this highlights a valuable role for recent woodlands in conservation of woodland species: several scarce and protected AWI species were found in recent woodlands, including *Hyacinthoides non-scripta*, *Lamiastrum galaebdolon*, and *Primula vulgaris*.

For the first time, the present study identified the separate and additive power of AWI and non-AWI species alpha richness to distinguish ancient and recent woodland. Although non-AWI richness was not a significant predictor in its own right, its inclusion created a superior ASNW-recent woodland model with higher classification accuracy. Higher AWI richness was significantly associated with ASNW, but non-AWI richness was not significant. These results are partly comparable with a prior study (Brunet et al. 2011), where woodland specialists correlated positively and significantly with woodland age, while woodland generalists were not significant, and species of open land correlated significantly and negatively. In the present study the non-AWI species subset comprised ruderal and some generalist species, while AWI species as a group include both specialist and generalist species (Brown et al., 2015).

The combined evidence of alpha and gamma richness reveals a limited number of non-AWI species present in ASNW. These are potentially a select group of non-AWI species with life traits akin to AWI species that permit establishment under ASNW conditions such as shade and low disturbance (De Keersmaeker et al., 2004; Sciama et al., 2009). Unlike the ruderal non-AWI species (such as *U. dioica* (De Keersmaeker et al., 2004)), this sub-group would not require a high nutrient status. AWI species colonisation of PAWS and recent woodland is well-canvassed in the literature (e.g. Atkinson et al, 2015, Baeten et al., 2009, Berges et al., 2017 Honnay et al., 1999; Jacquemyn et al., 2003), but far fewer studies have considered the reverse process of ruderal or non-woodland species colonisation in ASNW (e.g. Honnay, Verheyen, & Hermy, 2002). In contrast to AWI species, the strong dispersal ability of non-AWI species (Kimberley et al., 2013) suggests that colonisation inhibition is a greater limiting factor than dispersal. Further empirical research is needed to assess the ecological mechanisms underlying the distinctiveness of non-AWI assemblages in ancient woodland.

Beta diversity

The significant differences detected in AWI species composition between all comparisons of ASNW, PAWS, and recent woodland are echoed in a prior study where woodland species communities of ASNW differed significantly from plantations and stands undergoing restoration (Atkinson et al., 2015). The dissimilarity of AWI communities is fully attributable to compositional differences in the woodland types, as no significant differences in within-group heterogeneity were found (Warton et al., 2012). Acknowledged as poor dispersers, AWI species may not colonize recent woodland simultaneously, thus creating compositional differences between ASNW and recent woodland (Vellend et al., 2007), while differences between ASNW and PAWS are likely due to species loss in PAWS as a result of habitat change (e.g. Coote et al.. 2012).

These AWI compositional differences are further elucidated by the gamma and alpha metrics. AWI communities in ASNW, PAWS, and recent woodland are largely different permutations of species from the same pool, rather than due to particularly high species turnover (*sensu* Baselga, 2010). At the gamma scale, no distinct subset of frequently occurring AWI species was present in any of the three woodland types, which corresponds with several prior studies where no single AWI was entirely restricted to ancient woodland (Rose, 1999; Schmidt et al. 2014; Wulf, 2003). Additionally, the association of high alpha scale AWI richness with ASNW means that some species, whilst present in some PAWS and recent woodlands, do not occur as frequently. Such gaps in the species composition contribute to beta composition differences.

The distinctiveness of non-AWI species communities is a novel and largely unexplored facet of ASNW distinctiveness. However, there is seemingly no exact precedent for comparison. Although prior studies have surveyed non-AWI species, they have been incorporated within a total species beta analysis rather than separately tested (e.g. Berges et al., 2017; Coote et al., 2012). Non-AWI species are arguably stronger differentiators than AWI species using the beta metric due to significant differences in variance and slightly higher R^2 values.

The present study revealed three notable distinctions in non-AWI communities. Firstly, Non-AWI communities across ASNW sites were shown to be significantly more homogenous than for PAWS and recent woodland. This is also evident in the lower gamma richness of ASNW compared to PAWS and recent woodland. This high degree of consistency across a region is likely due to unsuitable abiotic conditions for early successional species within late successional stage continuity woodland (Cateau et al., 2015), as well as lack of niche availability for ruderals among well-established AWI populations. Secondly, there was no difference in mean species composition between ASNW and PAWS (despite the difference in variance) suggesting a common pool of non-AWI species associated with ancient woodland, regardless of replanting history. This finding reinforces the theory that PAWS retain some of the floristic characteristics of ASNW and have potential for restoration of not only AWI species but also the non-AWI component of the community (Coote et al. 2012; Palo et al., 2013). Thirdly, the species pool for non-AWI species in recent woodland was significantly different to both ASNW and PAWS, supporting the concept of potential reverse ancient woodland indicators (e.g. Webb and Goodenough, 2018).

Species associations

At the individual species level, *P. quadrifolia* was the only AWI significantly affiliated to ASNW. This species has been previously suggested to be one of the most strongly restricted to ASNW (Kirby & Morecroft, 2011; Hermy et al., 1999; Wulf, 1997) due its slow rhizomatous spread, long lifespan, and late maturation, which all require a low disturbance and long-continuity environment (Jacquemyn, Brys and Hutchings, 2008). Its preference for deep shade with an Ellenberg value of 3 (Hill, Preston, & Roy, 2004) further reduces the likelihood of establishing in recently restored PAWS or recent woodland.

The results revealed an important role for non-AWI species as reverse or negative indicators of ancient woodland. Increasingly, the application and reliability of traditional AWI lists has been questioned (Rotherham, 2011; Stone & Williamson, 2013; Webb & Goodenough, 2018). Five non-AWI species were identified as significantly associated with at least one woodland type compared to only one AWI. This finding supports a small number of earlier studies with significant results for negative indicators (Kelemen et al., 2014; Kirby & Morecroft, 2011; Wulf, 2003). Notably, the phosphate indicator, *U. dioica*, was significantly affiliated with recent woodland, likely due to increased soil phosphate associated with former agricultural land use (De Keersmaeker et al., 2004). High phosphate levels have been shown to indirectly hinder establishment of AWI species in recent woodland due competitive exclusion by ruderal phosphateophiles (Hermy, van den Bremt, & Tack, 1993).

Conclusions

This study presents a strong case for quantifying woodland biodiversity at a range of scales and extending ancient woodland vegetation appraisals to include the non-AWI species. These recommendations have practical applications in the identification and characterisation of ancient semi-natural woodlands, as well as for woodland conservation restoration, and creation. With further research, these can be considered for other UK regions and internationally. Accurate identification of ancient woodland has important planning and policy implications. Therefore, the use of notable negative or reverse indicators, as well as traditional AWI lists, is recommended when determining woodland continuity history. This study has shown additional value of non-AWI species across a range of scales and of individual species. Gamma AWI richness results supports conservation management of recent woodland, as sites are collectively capable of supporting similar AWI richness to ancient woodland and there is potential for those species to increase in prevalence under suitable conditions. The homogeneity of ASNW non-AWI communities is a noteworthy distinguishing factor. To assess success of PAWS restoration or new woodland creation projects, we recommend monitoring of non-AWI communities for increasing homogeneity in line with that of ASNW sites in the region in addition to the traditional AWI richness measure.

Acknowledgements

We sincerely thank the landowners and managers of study sites for access permission.

Data accessibility

http://eprints.glos.ac.uk/8345/

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Tables with legends

Figures with legends