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Identifying the trait syndromes of conservation indicator species: How distinct are British ancient woodland indicator plants from other woodland species?

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Abstract

Question

Ancient woodland indicator species (AWIs) are plant species which are thought to be restricted to areas of long continuity woodland habitat. In many cases however these species have been identified on the basis of personal, to some extent, subjective experience. Do the species proposed as AWIs according to these lists have traits in common and how distinct is their trait profile from that of other woodland plant species?

Location

United Kingdom

Methods

We applied classification tree analysis to a plant trait database to assess the extent to which proposed AWI species can be clearly separated from other woodland plants based upon their traits. We contrasted AWI species with an objectively defined list of plants that are not considered to be AWIs but that have been commonly recorded in woodlands. We also investigate the effects of phylogeny and region specificity on species' proposed AWI status.

Results

The results provide support for the distinctiveness of plant species thought to be associated with ancient woodland; they were found to be almost exclusively short, perennial species, usually with a high seed weight. Results also indicate that rarer AWIs have a more distinguishable trait profile than more common species. No link was found between phylogeny and AWI status.

Conclusions

AWI species do have a distinguishable trait profile, despite their often partially subjective selection. The results of the classification tree analysis suggest that traits

reflecting poor dispersal ability may be partly responsible for confining these species to ancient woodlands. This confirms other studies that emphasise their low ability to colonise secondary woodland sites and hence vulnerability to habitat conversion.

Keywords

Plant traits; classification tree; dispersal ability; phylogeny; rarity.

Nomenclature

Species nomenclature throughout is that of Stace (1997).

Running Head

Traits of ancient woodland indicator species.

1. Introduction

Ancient woodland indicator plants (AWIs) are vascular plant species that are considered to be restricted to areas of long-established woodland habitat. Since they were first proposed as a method of assessing the conservation value of woodland in Lincolnshire by Peterken (1974), lists of plants which are considered AWIs in other regions of Europe and North America have been developed (e.g. Honnay et al. 1998, Motzkin et al. 1999, Verheyen et al. 2003).

Areas of ancient woodland, as defined by Peterken (1977), are considered a conservation priority due to their ability to sustain a large number of rare or vulnerable species that are unlikely to colonise isolated younger woodland (Peterken & Game, 1984). They may also act as refuges for species dependent on habitat types associated with low farming intensity (Smart et al. 2006). As such, there have been efforts to map remaining ancient woodland habitat (Goldberg et al. 2007) and to protect some of these areas, for example in the UK through notification as Sites of Special Scientific Interest and Priority Habitats under the UK Biodiversity Action Plan (BRIG, 2008). AWI species provide a useful means with which to identify ancient woodland and a simple tool to help assess woodland diversity and gauge the continuity of woodland cover, although they should be used in conjunction with historical land use data (Spencer & Kirby, 1992).

Despite the conservation importance of ancient woodland and the use of indicator species in identifying such habitats, concerns remain over the way in which species have been designated as AWIs, often based upon anecdotal evidence of their association with ancient forest (Rolstad et al. 2002). Furthermore, few indicator species are entirely restricted to ancient woodland (Wulf, 2003), meaning that a subjective decision must be taken as to which species occur too frequently outside ancient woodland habitat to be considered AWIs. Too stringent a set of requirements and the resulting list of indicators will be too short to be useful, too loose a definition of an AWI and less specialised plant species may reduce the effectiveness of the indicators chosen (Rose, 1999).

Here we test whether lists of species suggested as AWIs for different parts of Britain, often defined at least partly in a subjective way, do have distinctive traits such that they might be considered as a guild of woodland specialists. An objective classification tree method was used to explore differences between species that are currently proposed as AWIs compared to non-AWI species by identifying fundamental life-history traits that can be used to separate species from the two groups.

Previous studies have found differences in Ellenberg indicator values between AWI and non-AWI species, with AWIs preferring low light conditions with soils of intermediate nitrogen concentration and wetness (Hermy et al. 1999). However, these Ellenberg values do not represent morphological or behavioural traits and hence offer limited insight into the mechanisms of dispersal, establishment and persistence that define AWI species.

The distribution of species associated with ancient woodland habitat has been shown to be limited by dispersal ability and longevity (Wulf, 2003; Hermy & Verheyen, 2007). Short species with heavy seeds are thought to have lower ability to colonise new habitat and adapt to land-use change (Verheyen et al. 2003; Hermy & Verheyen, 2007). Consequently we hypothesise that dispersal-related traits such as seed terminal velocity and seed weight are likely to prove important factors that can be used to group AWI species together. Due to the shade tolerance of AWI species and their association with low to moderate macro-nutrient availability, specific leaf area (SLA) was also expected to differ between AWIs and non-AWIs. While high SLA has been associated with shade tolerance (Hodgson et al 2011) it is also strongly associated with productive, human modified habitats. High SLA therefore may only be an effective predictor of AWI status after taking into account the presence of other trait states that differentiate species along the productivity and land-use intensity gradient.

When analysing the explanatory power of multiple traits across many species, it is important to consider the fact that phylogenetic relatedness may result in non-independence between species due to covariance among traits other than those included in the analysis (Felsenstein, 1985). Using phylogeny as an explanatory framework reduces the likelihood of misinterpreting ecological patterns that are driven by common ancestry. AWI species may be largely restricted to certain taxonomic groups. If this is the case, the phylogeny of these species may confound any attempt to separate AWIs from non AWIs based upon specific traits. To investigate the possibility that AWI species can be differentiated as effectively by their ancestral relatedness as by the chosen traits, we performed a second, separate analysis which also attempted to split proposed AWI species from non AWIs, in this case based solely upon their phylogeny.

In Britain AWIs can be indicators of ancient woodland across the whole of their range or only considered such in certain regions, despite being distributed much more widely (Kirby, 2006). For example, some species may only be classified as AWIs in relatively more intensively-managed landscapes because ancient woodlands provide the only remaining favourable niche space. The same species may however be more common in semi-natural habitats in less intensively-managed regions, and hence not considered AWIs in these regions because they are evidently not restricted to ancient woods. This wider niche breadth may therefore correspond with a trait profile less readily discriminated from other non-AWI species that occur in the same mid or early successional habitats.

We therefore hypothesise that species that are considered AWIs in only a small number of local areas despite being widely distributed across many regions have a less distinctive, more generalist set of traits than those which are AWIs across the whole of their range. This should make them harder to separate from the non-AWI species pool. Conversely, species may only be AWI in a subset of regions because they are rare. Rarer AWIs may have an even more distinctive trait profile if the reason for their rarity is the possession of specialised trait combinations that are associated with restriction to ancient woods.

In this paper we test the hypothesis that proposed AWI species can be clearly separated from non-AWI woodland species on the basis of traits linked to poor dispersal and adaptation to low light availability during the peak growing season. Having determined the trait differences between the two groups, we test two hypotheses about the trait profiles of AWI species that are indicators only in certain regions. First, that regional AWIs are less distinguishable from non-AWIs than pan-national AWIs. Second, that those regional AWIs are more distinguishable from non-AWIs but only where they are rare across Britain. Better knowledge of the different sets of traits that are associated with AWI species should provide improved understanding of why their distribution is restricted to ancient woodland and help to develop more effective measures to identify and conserve their habitat in the future. Trait analysis might also suggest other species that might be investigated as possible ancient woodland indicators.

2. Material and methods

2.1 Classification and regression tree analysis

Classification and regression tree (CART) methods (Breiman et al. 1984) are a set of analytical techniques that can be used to explore and model large sets of data. Their ability to consider interactions between variables and to deal with missing values make them well suited for modelling complex ecological datasets (De'ath & Fabricius, 2000). Here, CART analysis was performed on a database of information on the life history traits of British woodland plant species, using the "rpart" add-on (Therneau, Atkinson & Ripley, 2012) in the statistical software R (R Development Core Team, 2011).

CART models are built by applying a series of splits to an input dataset. At each split the data is divided into two groups based upon the value of the explanatory variable (in this case the plant trait) that results in the groups produced being as uniform as possible in terms of the response variable (here species' proposed AWI status). By applying this method to the plant species data a tree model was produced that identifies differences between the traits of the proposed AWI species and other woodland plants (Figure 1). The extent to which the CART model was able to separate the AWIs from non-AWIs at each split also provided a way of assessing the strength of differences between the two groups of species for each trait, as well as the extent to which the proposed AWI species share common characteristics. In order to further investigate the way in which the tree model used the plant traits to group species as either AWI or non-AWI, the final node into which each species was classified was also extracted from the model (see Appendix 1, Table 1).

The usual procedure in CART modelling is to fit an overly large (and therefore overfitted) tree model and then prune this back to its optimal level of complexity according to assessment of the cross-validated error (Breiman et al. 1984). Here this was achieved by carrying out 50 sets of tenfold cross-validation and taking an average of the mean cross-validated error of each sized tree, following the method recommended by De'ath & Fabricius (2000). This information was then used to determine the level of tree complexity that provided the lowest mean cross-validated error (here a tree with eight splits). The complexity parameter associated with this size of tree (0.028) was then used in rpart to prune the full tree to its optimal size and produce the classification tree model (Breiman et al. 1984). The control settings used for the fitting function in rpart; the minimum number of observations in a node before attempting a split and the minimum number of observations in a terminal node, were set at 20 and 5 respectively. Changing these settings had little effect on the pruned tree model. Surrogate variables were used where trait data were missing for a particular split, using data for other variables to estimate the missing values (Breiman et al. 1984). If all potential surrogates were missing then species were prevented from continuing through the model rather than being sent in the majority direction (as is the default in rpart). In this case sending observations the way of the majority would have biased the model in favour of non-AWIs, particularly since AWIs had a higher proportion of missing data.

Table 1. Summary of input variables used to fit the classification tree model.

Trait	Variable type	Possible categories and ranges of values	No. missing values	
			AWI (n = 138)	Non-AWI (n = 423)
Maximum height	Continuous	4-5800 centimetres	0	0
Lifespan	Categorical	Perennial/biennial/ annual	0	0
Growth form	Categorical	Woody species/grass/sedge/ forb/fern/other monocotyledon	0	0
Seed weight (weight of 1000 seeds)	Continuous	0.001-12980 grams	45	66
Seed terminal velocity	Continuous	0.110-5.42 metres per second	66	151
Specific leaf area	Continuous	3.64-86.10 millimetres squared per milligram	54	35
Seed bank persistence	Categorical	Transient seeds/seeds persist for a short time/some persistent seeds/large bank of persistent seeds all year round	39	0
Dispersed by wind	Boolean	True/false	43	0
Dispersed by water	Boolean	True/false	43	0
Dispersed by animal vector	Boolean	True/false	43	0
Dispersed by human vector	Boolean	True/false	43	0

2.2 Testing for effects of phylogeny

In order to test for relationships between species’ phylogeny and their AWI status a second CART analysis was performed. This involved using molecular phylogentic data on the genus, family and order of 1888 British plant species, taken from PLANTATT (Hill, Preston & Roy, 2004). These phylogenetic factors were used as explanatory variables in a classification tree model, which attempted to distinguish AWIs from non AWIs. The methods used to build and prune the tree model were those described in section 2.1. The accuracy with which this model was able to classify these species provided a way of assessing the strength with which AWI status is linked to phylogeny, and therefore whether variation in AWI status can be reliably attributed to species' traits.

2.3 Effects of rarity and regional AWI status

The classification tree analysis grouped proposed AWI species into one of two categories based upon their traits; either identifying them as potential AWIs or as non-AWIs. It was predicted that the probability of an proposed AWI species being identified as an AWI would increase with species' rarity, since rarer AWIs were expected to have a more distinct trait profile. However, species commonness and assignment as AWI only in local regions should reflect a more generalist trait profile therefore associated with a greater chance of being classified as a non-AWI. We used multiple logistic regression in the R package MASS (Venables & Ripley, 2002) to test the hypothesis that the probability of proposed AWIs being correctly classified by the tree model was related to their rarity and the number of regions for which they are AWIs. Species' AWI status in various areas of Britain; Derbyshire, Lincolnshire, Carmarthen, North Yorkshire, Dorset, Worcestershire, Somerset and Angus is documented in Kirby (2006) and a count of the number of these (eight) regions in which each species is considered an AWI was used in the analysis. Species' rarity was determined from PLANTATT (Hill, Preston & Roy, 2004) and measured as number of occurrences in British 10 km squares in the period 1987-1999. The interaction between rarity and number of AWI regions was also included in the model. Due to the degree of intercorrelation between rarity and number of regions a type III likelihood ratio test was carried out to determine the significance of the explanatory variables. This prevented the order in which variables were entered into the model affecting the results. Out of the 138 AWI species used in the CART analysis, 108 were included in the logistic regression, leaving out 29 AWI species unclassified by the tree model due to lack of data and one species for which information on regional AWI status was not available.

2.4 Plant species data

The species used in the classification tree analysis included 138 that had been proposed as ancient woodland indicator plants (AWIs) in at least part of Britain, based on the list collated by Kirby (2006) and 423 other woodland species not considered ancient woodland indicators (non-AWIs) but recorded in quadrats located in woodland as part of the 2007 Countryside Survey of Great Britain (Norton et al. 2012). This approach enabled the use of randomly sampled representative data for woodlands across Britain to define a species pool of non-AWIs that nevertheless occur in woodland habitat. Crucially this reduced the extent to which differences between the traits of AWIs and non-AWIs were obscured by trait differences linked to species preferences for non-woodland habitats. The list of AWIs used was created by combining twelve existing lists of proposed indicators across Britain drawn up by numerous authors, as described in Kirby et al. (2012). Although a number of the species on these lists were proposed as AWIs based upon independent data showing their association with ancient woodland, some have been assessed based only upon the judgement of the expert surveyors. By comparing the traits of these proposed

AWIs with those of other woodland species we aim to establish whether these species do have a different set of characteristic traits and thus are a useful conservation tool.

Eleven plant traits were used to build the classification tree model (Table 1), representing those life history attributes considered most likely to differ between AWIs and non-AWIs. This included various dispersal related traits; seed weight, seed terminal velocity and maximum recorded species height (Soons et al. 2004, Thomson et al. 2011). A number of categorical variables were included in the model, relating to species' ability to use a number of dispersal vectors. Species could be assigned more than one dispersal vector; for example a species could be considered both wind and water dispersed. Since recent work suggests that dispersal vector variables based upon seed morphology are in fact weak predictors of the actual ability of species to disperse through the landscape (Tackenberg et al. 2003; Eycott et al. 2007) we expected that these variables would not be successful predictors of AWI status of woodland plants.

In addition to the dispersal centred traits, data on species' lifespan, seedbank persistence, growth form and specific leaf area (SLA) were also used in the classification model. SLA in particular has been shown to be a key trait in determining plant species' resource use strategy (Westoby, 1998) and is also correlated with a number other traits such as growth rate, leaf lifespan and leaf nitrogen content (Reich et al. 1997). Together these traits therefore represented a number of the competitive and shade tolerant strategies likely to differ between AWIs and non AWI species.

The trait information was obtained from the Electronic Comparative Plant Ecology database (Grime et al. 1995), the LEDA traitbase (Kleyer et al. 2008) and other reference materials including Stace (1997) and PLANTATT (Hill et al. 2004). Where species' dispersal vectors were not available they were inferred from relevant literature and by inspection of plant parts in the illustrations of the British Flora (Ross-Craig, 1948-74).

Although efforts were made to minimise gaps in the database through obtaining information from as many sources as possible, the difficulty in obtaining trait data for all species meant that a number of missing values were still present in the database (Table 1). One advantage of CART techniques is their ability to handle missing values without entirely removing incomplete records from the model; however rates of misclassification may be higher for traits with a large number of missing values such as seed terminal velocity due to the lower amount of information present.

3. Results

3.1 Trait analysis

The final classification tree model (Figure 1) retained six of the plant trait variables tested; seed weight, seed terminal velocity, maximum species height, lifespan, growth form and specific leaf area. None of the four dispersal vector variables nor seedbank persistence were used by the tree model to discriminate between AWI species and non-AWIs, although the effect of these traits may be represented by some of the other variables, for example through the continuous variables describing seed characteristics.

The tree model firstly separated ferns and other monocots (59 species, largely geophytes with underground storage organs) from other growth forms. The AWI status of the former group was best reflected by their seed terminal velocity; those with fast falling seeds were classified as AWIs, those with slow falling seeds as non-AWIs (Node 2, Figure 1). At this node only 7 proposed AWIs were classed as non AWI species.

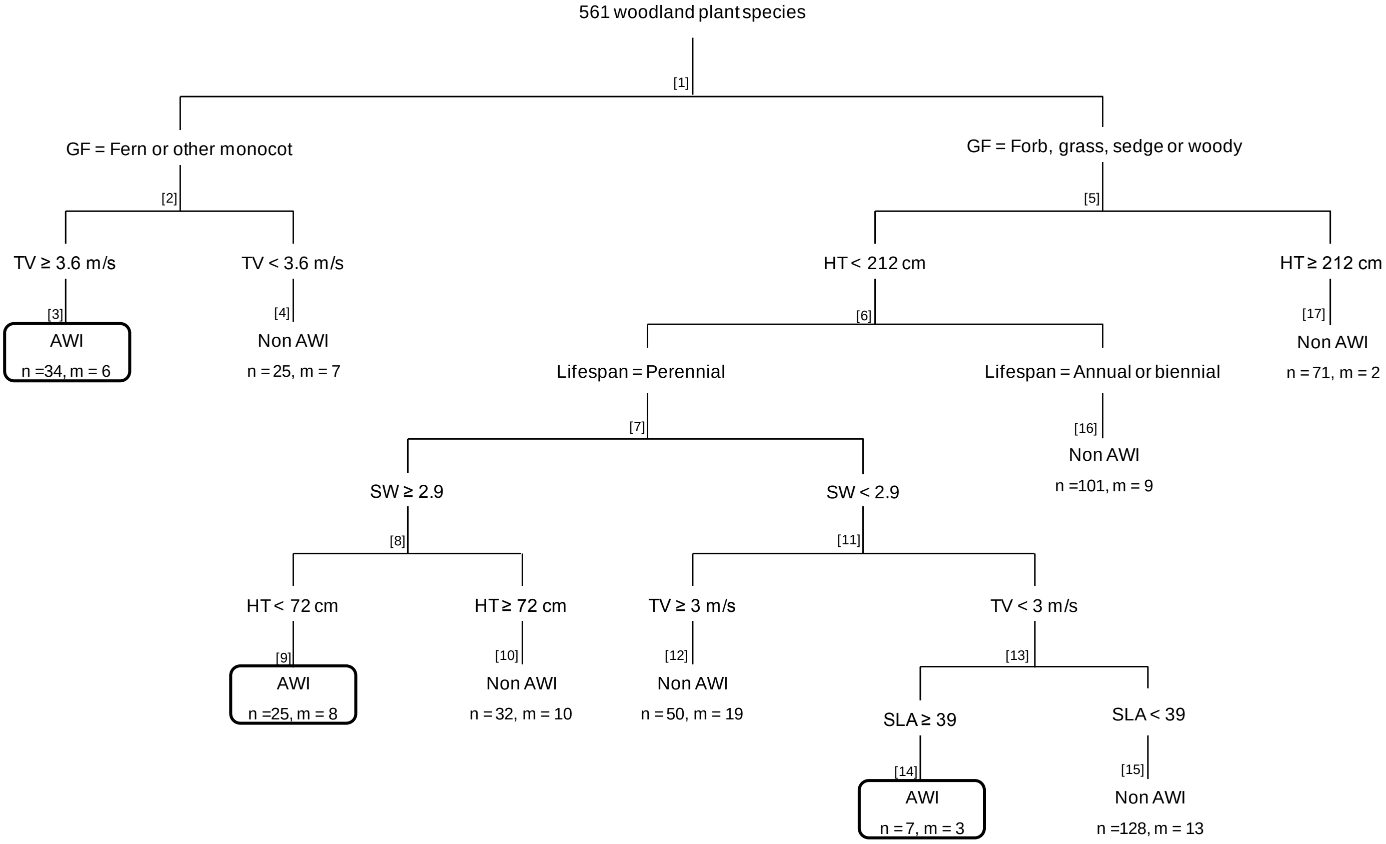

Figure 1. Classification tree model showing how different plant trait variables contribute to species' AWI status. Split abbreviations; GF = growth form, TV = seed terminal velocity, SLA = specific leaf area, SW = seed weight, HT = maximum height. Node labels are given in square brackets and can be cross-referenced to the species

lists in the appendix (Appendix 1, Table 1). n = number of species within each terminal node, m = number of species misclassified at each terminal node.

In other growth forms (forbs, grasses, sedges and woody species) tall species were not considered to be AWIs. Only two proposed AWI species had a maximum height of greater than or equal to 212 cm, causing them to be classified as non-AWI species according to the tree model (Figure 1). Among those plants shorter than 212 cm, most annual and biennial species were classified as non-AWI species, with 9 proposed AWIs terminating in this node, out of 101 species in total. Of the remaining species (perennial forbs, grasses, sedges and woody species shorter than 212 cm), species with light, slow falling seeds were not classified as AWIs unless they had an extremely large SLA. Species with heavy seeds were classified as AWIs if shorter than 72 cm but not if taller than 72cm.

88 species were not classified due to missing values; 29 AWI species and 59 non-AWIs. The traits that most clearly distinguished the two groups were height and lifespan; these two splits identifying 161 non-AWI species, while only including 11 proposed AWI species. The least certain group, node number 12 on Figure 1, contained species with relatively light, fast falling seeds. This group contained almost equal numbers of both proposed AWIs and non-AWIs.

3.2 Phylogeny and AWI status

When the genus, family and order of plant species were used to predict their AWI status, the resulting classification tree did not retain any of the three explanatory variables; an optimal tree model was returned which contained no splits. Including the phylogenetic variables in this model only resulted in the cross-validated error of the tree increasing. This provides strong evidence that phylogeny is not an effective predictor of species AWI status.

3.3 Regional AWIs

Results of the logistic regression found no significant relationship between the number of regions for which a species was considered an AWI and its probability of misclassification (Chi squared = 0.0506, p = 0.82200). The interaction between rarity and number of regions was also non-significant (Chi squared = 1.0808, p = 0.29853). Rarity on its own however did have a significant effect, with rarer AWI species more likely to be correctly classified by the tree model (Chi squared = 4.4219, p = 0.03548).

4. Discussion

The results of the CART analysis largely support the hypothesis that dispersal-related traits are useful in discriminating AWIs from other plant species found in woodlands. Maximum species height, seed weight and seed terminal velocity all emerged as key correlates with AWI status. Phylogeny was found to have no influence on species' AWI status, with none of genus, family or order being able to predict species AWI status successfully. This indicates that AWIs are not confined to a particular group of related species, rather being spread across a wider range of taxa. Since none of the phylogenetic variables were capable of discriminating successfully between AWI species and non AWIs, it is unlikely that the discriminating power of the traits analysed here is confounded by the common ancestry of these species. Hence these traits seem to be those which best explain the restriction of many proposed AWI species to ancient woodlands.

Small stature, found in almost all AWI species, is associated with a number of strategies for tolerating low light throughout much of the growing season (Westoby, 1998). Vernal species are constrained to complete seasonal leaf production and flowering in the narrow window between unfavourable spring temperatures and canopy leafing after which carbon fixation and biomass production is strongly light-limited (Augspurger et al. 2005). Survival for these species may therefore centre on tolerating or avoiding shade rather than growing woody biomass. Where light (or another resource) is less limiting, taller species, identified almost exclusively as non-AWIs, may have the competitive advantage.

AWI plants tend to be perennial species with heavy seeds; traits which other studies have linked to poor colonising ability (Verheyen et al. 2003). Low dispersal ability is thought under some conditions to reduce the ability of species to form viable metapopulations, leading to higher vulnerability to habitat loss and fragmentation and slower response to changes in landscape structure (Fischer & Lindenmayer, 2007). The delayed response to landscape change shown by many perennial forest plants can lead to an extinction debt forming in disturbed areas, with a number of existing species destined for eventual extinction under the modified conditions (Eriksson, 1996; Kuussaari et al., 2009). Many AWIs in fragmented habitat patches may therefore exist as part of such remnant populations and consequently be at risk of future extinction from such habitat.

As predicted, the dispersal vector variables were not useful in discriminating between AWIs and other woodland plants. This is likely due to the poor ability of such categorical variables based upon seed morphology to reflect observed dispersal rates of plant species (Tackenberg, 2003).

In the classification tree model, traits such as growth form, lifespan and height provided an effective initial separation between proposed AWIs and non-AWI species, suggesting that the two groups tend to have distinct values for these characteristics. Higher misclassification rates at nodes lower down in the tree model may occur because important discriminating information has not been included, either because the values for included traits are missing or because key traits have not been included. However it may also mean that what is important in determining AWI status is the interaction between the plant traits and their landscape context. For example if all that is asked of an AWI is that it occurs much less in secondary woodland than in ancient woodland this could still be consistent with a species occurring in a range of low-productivity mid-successional habitats (e.g. Motzkin et al 1999). Species that are less likely to occur in secondary woodland but can occur in other non-woodland habitats of long continuity include those in node 4, such as the fern *Oreopteris limbosperma* and the horsetails *Equisetum sylvaticum* and *E.telmateia*. These species are predicted by the tree model to be non-AWIs since they have low seed terminal velocity (Figure 1; Appendix 1, Table 1) and are widespread in Britain, occurring on linear features such as road verges, streamsides and hedge banks, especially in the more oceanic west and north. They are not however typical of the productive, disturbed conditions that often persist as abiotic legacy effects within secondary woodland (Gilliam, 2007). These species may therefore still be valid AWIs where their relative abundance in ancient rather than secondary woodland is more important than their absolute restriction to woodland. Other species where this applies include *Geranium sylvaticum* and *Stachys officinalis*, both of which are considered AWIs, but also occur outside the woodland environment in unimproved hay meadows, and *Cardamine amara, Conopodium majus, Hypericum tetrapterum* and *Wahlenbergia hederacea* which occur widely in non-woodland habitats but where they do occur in woodland this is more likely to be of long continuity than secondary.

A number of widespread species (for example at node 9, *Cruciata laevipes, Ranunculus ficaria, Symphytum tuberosum* and *Viola hirta*) associated with linear features and were predicted to be AWI based on their trait sets. The management of such features often involves infrequent pulse disturbance such as cutting that sets back succession creating disturbance regimes and abiotic conditions that resemble those of woodland gaps. Short perennial herbs with limited seed dispersal in space or time are also characteristic of long-established meadows and pastures (Hodgson & Grime, 1990) and hence such species might be classed as having AWI type traits. Examples include *Cirsium acaule* and *Sanguisorba minor* (node 9; Appendix 1, Table 1) both short perennials of grazed calcareous grassland and best considered as outliers within the woodland species pool analysed. Adding in further traits related to shade tolerance, along with traits that could discriminate grazing tolerance might have allowed better separation of these species (Pakeman, 2004).

Preferences of some AWIs for non-woodland habitats may also mean that species are only considered indicators in regions where the non-woodland habitat in which they are found elsewhere in Britain is absent. The situation is however complicated for species such as *Hyacinthoides non-scripta* where the range of habitats they can occupy changes geographically as a function of temperature and not necessarily habitat availability (Blackman & Rutter, 1954). Moving toward the western fringes of the British Isles, mean minimum winter temperatures increase and this frost-sensitive species becomes increasingly common in mid-successional habitats.

Node 15 comprised a large, well-differentiated group of perennial herbs with light, slow falling seeds; likely to be more widely dispersed than the typical AWI (Appendix 1, Table 1). Most were predicted to be non-AWI but a subset of proposed AWIs were predicted to be non-AWI, including *Carex acutiformis, C.remota, Fragaria vesca* and *Scrophularia nodosa*. All are either grazing intolerant or not favoured by high productivity and so likely to find woodland a favourable refuge. Their wide distribution may however make them less reliable as AWIs.

Rarity was found to have a significant effect on whether or not a proposed AWI species was considered to possess AWI-like traits by the tree model. The rarity of these species may be due to highly specialised sets of traits, such as preference for high levels of shade and infrequent disturbance, which confine them to a narrow range of conditions. These species are likely to be more dependent on ancient woodland habitat and therefore more distinct from other woodland plants with a more general set of traits and consequently looser association with old growth forest.

Other characteristics may differentiate between AWIs and other woodland plants but for which trait data were not available. For example the amount of nuclear DNA that a species possesses is associated with a number of plant traits such as shade tolerance, phenology and generation time (Bennet, 1987) and as such might prove effective in distinguishing AWIs from other woodland plants. Growth rate may also be important, since plants with shade tolerant strategies have lower rates of growth (Coley, 1988) thus typical AWI species may have slower growth than non-AWI plants. Inclusion of relative growth rate in the classification tree model may have been able to improve the rate of successful classification but we would expect the discriminatory power associated with this trait to have been captured by specific leaf area given the strong correlation between the two.

5. Conclusions

Clear trait-based patterns emerged from the CART modelling, suggesting that a distinct trait profile is associated with AWI species: despite many lists being at least partly based on subjective assessments they do appear to be a distinct guild of plants. In summary an AWI species is most likely to be a short perennial with heavy, fast falling seeds; often poorly dispersing species, not favoured by intensive disturbance regimes and high productivity. Such a step constitutes a useful generalisation that subsumes taxonomic identity and should aid further understanding of the mechanisms that confine these species to older woodlands. This knowledge may help better parameterise models of landscape connectivity for resilience mapping (e.g. Vos et al. 2008).

The functional distinctiveness of AWI species provides some support for the use of such species as a group to identify areas of conservation importance. However we also found trait-based similarities between many AWI species and non-AWIs that are found in rarer, less frequently disturbed semi-natural habitats. Some of these might merit further investigation to see if they might also be AWI where they occur in woodland.

The strength of the association between these AWIs and ancient woodland habitat depends on landscape context. This should be considered when using the presence or absence of such indicator species to assess the conservation importance of woodland habitat. Rarer AWI species were more clearly discriminated from non-AWI woodland species on the basis of their traits and as such these species may be most reliable as indicators of ancient woodland.

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7. References

Augspurger, C.K., Cheeseman, J.M. & Salk, C.F. Light gains and physiological capacity of understorey woody plants during phonological avoidance of canopy shade. *Functional Ecology* 19: 537-546.

Bennet, M.D. 1987. Variation in genomic form in plants and its ecological implications. *New Phytologist* 106: 177-200.

BRIG (Biodiversity Reporting and Information Group) (ed. A. Maddock). 2008. *UK Biodiversity Action Plan Priority Habitat Descriptions*. UK Biodiversity Partnership. http://jncc.defra.gov.uk/PDF/UKBAP_PriorityHabitatDesc-Rev2011.pdf (PDF, 1.3Mb). Accessed 13/12/2012.

Blackman, G.E. & Rutter, A.J. 1954. Endymion nonscriptus (L.) Garcke. *Journal of Ecology* 42: 629-638.

Breiman, L., Friedman, J., Olshen, R. & Stone, C. 1984. *Classification and regression trees*. Wadsworth International Group, Belmont, California.

Coley, P.D. 1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia* 74: 531-536.

De’ath, G. & Fabricius, K.E. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* 81: 3178-3192.

Eriksson, O. 1996. Regional dynamics of plants: a review of evidence for remnant, source-sink and metapopulations. *Oikos* 77: 248-258.

Eycott, A.E., Watkinson, A.R., Hemami, M.R. & Dolman, P.M. 2007. The dispersal of vascular plants in a forest mosaic by a guild of mammalian herbivores. *Oecologia* 154: 107-118.

Felsenstein, J. 1985. Phylogenetics and the comparative method. *American Naturalist* 125: 1-15.

Fischer, J. & Lindenmayer, D.B. 2007. Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* 16: 265-280.

Gilliam, F.S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *BioScience* 57: 845-858.

Goldberg, E., Kirby, K., Hall, J. & Latham, J. 2007. The ancient woodland concept as a practical conservation tool in Great Britain. *Journal for Nature Conservation* 15: 109-119.

Grime, J.P., Hodgson, J.G., Hunt, R. & Thompson, K. 1995. *The Electronic Comparative Plant Ecology.* London. Chapman & Hall.

Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C., Lawesson, J.E. 1999. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation* 91: 9-22.

Hermy, M. & Verheyen, K. 2007. Legacies of the past in the present-day forest biodiversity: a review of past land-use effects on forest plant species composition and diversity. *Ecological Research* 22: 361-371.

Hill, M.O., Preston, C.D. & Roy, D.B. 2004. *PLANTATT - attributes of British and Irish Plants: status, size, life history, geography and habitats*. Centre for Ecology and Hydrology, Huntingdon.

Hodgson, J.G. & Grime, J.P. 1990. The role of dispersal mechanisms, regenerative strategies and seedbanks in the vegetation dynamics of the British landscape. In: *"Species Dispersal in Agricultural Habitats"* Eds; Bunce, R.G.H., Howard, D.C. Institute of Terrestrial Ecology. Belhaven Press, London, 65-81.

Hodgson, J.G., Montserrat-Marti, G., Charles, M., Jones, G., Wilson, P., Shipley, B., Sarafi, M., Cerabolini, B.E.L., Cornelissen, J.H.C., Band, S.R., Bogard, A., Castro-Diez, P., Guerrero-Campo, J., Palmer, C., Perez-Rontome, M.C., Carter, G., Hynd, A., Romo-Diez, A., de Torres Espuny, L. & Royo Pla, F. 2011. Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Annals of Botany* 108: 1337 - 1345

Honnay, O., Degroote, B. & Hermy, M. 1998. Ancient forest plant species in western Belgium: A species list and possible ecological mechanisms. *Belgian Journal of Botany* 130: 139-145

Kirby, K. 2006. Ancient Woodland Indicator (AWI) plants, in Rose, F. (ed) *The wildflower key*. Penguin Group, London.

Kirby, K.J., Pyatt, D.G. & Rodwell, J. 2012**.** Characterization of the woodland flora and woodland communities in Britain using Ellenberg Values and Functional Analysis. In *Working and Walking in the Footsteps of Ghosts: volume 1 the wooded landscape',* edited by I D Rotherham, M Jones, C Handley, Wildtrack Publishing, Sheffield pp66-86.

Kleyer M., Bekker R.M., Knevel, I.C., Bakker, J.P., Thompson, K.Sonnenschein, M., Poschlod, P., Van Groenendael, J.M., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Götzenberger, L., Hodgson, J.G., Jackel, A-K., Kühn, I., Kunzmann, D., Ozinga, W.A., Römermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J., Tackenberg, O., Wilmann, B., Cornelissen, J.H.C., Eriksson, O., Garnier, E. & Peco, B. 2008. The LEDA traitbase: a database of life-history traits of the NW European flora. *Journal of Ecology* 96: 1266-1274.

Kuussaari, M., Bommarco, R., Heikkinen, K., Helm, A., Krauss, J., Lindborg, R., Ockinger, E., Partel, M., Pino, J., Roda, F., Stefanescu, C., Teder, T., Zobel, M., & Steffan-Dewenter, I. 2009. Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology & Evolution* 24: 564-571.

Motzkin, G., Wilson, P., Foster, D.R. & Allen, A. 1999. Vegetation Patterns in Heterogeneous Landscapes: The Importance of History and Environment. *Journal of Vegetation Science* 10: 903-920.

Norton, L.R., Maskell, L.C., Smart, S.S., Dunbar, M.J., Emmett, B.E., Carey, P.D., Williams, P., Crowe, A., Chandler, K., Scott, W.A. & Wood, C.M. 2012. Measuring stock and change in the GB countryside for policy – key findings and developments from the Countryside Survey 2007 field survey. *Journal of Environmental Management* 113: 117-127.

Pakeman, R.J. 2004. Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *Journal of Ecology* 92: 893-905.

Peterken, G.F. 1974. A method for assessing woodland flora for conservation using indicator species. *Biological Conservation* 6: 239-245.

Peterken, G.F. 1977. Habitat conservation priorities in British and European woodlands. *Biological Conservation* 11: 223-236.

Peterken, G.F. & Game, M., 1984. Historical factors affecting the number and distribution of vascular plant species in the woodlands of central Lincolnshire. *Journal of Ecology* 72: 155-182.

R Development Core Team, 2011. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Reich, P.B., Walters, M.B. & Ellsworth, D.S. 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences* 94: 13730-13734.

Rolstad, J. Gjerde, I., Gundersen, V.S. & Sætersal, M. 2002. Use of indicator species to assess forest continuity: a critique. *Conservation Biology* 16: 253-257.

Rose, F. 1999. Indicators of ancient woodland - the use of vascular plants in evaluating ancient woods for nature conservation. *British Wildlife* 10: 241–251

Ross-Craig, S. 1948-74. *Drawings of British Plants, Vols 1-8*. Bell, London.

Smart, S.M., Marrs, R.H., Le Duc, M.G., Thompson, K., Bunce, R.G.H., Firbank, L.G. & Rossall, M.J. 2006. Spatial relationships between intensive land cover and residual plant species diversity in temperate farmed landscapes. *Journal of Applied Ecology* 43: 1128-1137.

Soons, M.B., Heil, G.W., Nathan, R. & Katul, G.G. 2004. Determinants of long-distance seed dispersal by wind in grasslands. *Ecology* 85: 3056–3068.

Spencer, J.W., Kirby, K.J. 1992. An inventory of ancient woodland for England and Wales. *Biological Conservation* 62: 77-94

Stace, C. 1997. New Flora of The British Isles. Cambridge: Cambridge Univ. Press

Tackenberg, O., Poschlod, P., Bonn, S. 2003. Assessment of Wind Dispersal Potential in Plant Species. *Ecological Monographs* 73: 191-205.

Therneau, T.M. & Atkinson, B. (R port by Ripley, B.). 2012. *rpart: Recursive Partitioning*. R package version 3.1-51. http://CRAN.R-project.org/package=rpart

Thomson, F.J., Moles, A.T., Auld, T.D. & Kingsford, R.T. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. *Journal of Ecology* 99: 1299–1307.

Venables, W.N. & Ripley, B.D. 2002. *Modern Applied Statistics with S. Fourth Edition.* Springer, New York.

Verheyen, K., Honnay, O., Motzkin, G., Hermy, M. & Foster, D.R. 2003. Response of forest plant species to land-use change: a life-history approach. *Journal of Ecology* 91: 563-577.

Vos, C.C., Berry, P., Opdam, P., Baveco, H., Nijhof, B., O’Hanley, J., Bell, C. & Kuipers, H. 2008. Adapting landscapes to climate change: examples of climate proof ecosystem networks and priority adaptation zones. *Journal of Applied Ecology* 45: 1722-1731.

Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* 199: 213-227

Wulf, M. 2003. Preference of plant species for woodlands with differing habitat continuities. Flora - Morphology, Distribution, *Functional Ecology of Plants* 198: 444-460

List of appendices

Appendix 1: Table of the 561 plant species used in the CART analysis.