Investigating local distribution and abundance of common cow-wheat *Melampyrum pratense* in the Blean Woods National Nature Reserve, Kent.

by

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Contents

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ABSTRACT

The pre-diapause larvae of the rare heath fritillary *Melitaea athalia* in the woodlands of southern England utilise common cow-wheat *Melampyrum pratense* as their primary larval host plant (LHP). Conservation of the heath fritillary therefore depends on the presence and management of common cow-wheat (CCW) in its habitat. For this study, an investigation on the variables affecting the local distribution and abundance of CCW in the Blean Woods National Nature Reserve (NNR), Kent was carried out. Thirty $100m^2$ grid squares across the Blean Woods NNR were assessed for eight variables thought to affect CCW distribution and abundance. These variables included distance to ant nests, distance to oak trees, light intensity, canopy cover, bare ground cover, soil pH, soil calcium content and oak sapling distribution. A principal component analysis (PCA) incorporating the eight measured variables for each grid square was used to identify the principal components that explain the variation in the data of CCW % cover. The results of the PCA were then used to reduce the number of variables used in stepwise regression and multiple regression models to then identify variables significantly associated with CCW % cover per grid square.

The results showed that oak sapling cover in a sampled grid square was the only variable among those recorded that had a significant effect on CCW distribution and abundance. This could be due to CCW utilising oak saplings in a hemiparasitic manner or simply a case of the species having similar environmental preferences. The statistical analysis also revealed that CCW seemed to be distributed non-randomly and in a patchy pattern across most of the grid squares. In addition, a comparison of CCW cover in grid squares based on abundance records from 2013 and 2018 showed that there seems to have been no significant change of CCW cover in these grid squares during the intervening five-year period.

This study suggests that CCW may have some hemiparasitic relationship with oak saplings, but due to its non-random distribution and gradual dispersal, needs long-term management to increase both distribution and abundance to aid in the conservation of the heath fritillary. This would be best supported by ensuring that habitat suitable for CCW remains stable and well-connected throughout the Blean. Recommendations are made for further study within other areas of the wider Blean complex in East Kent and in Essex with more localised studies into CCW distribution required in areas where the heath fritillary is present.

1. INTRODUCTION

Species conservation often hinges on the conservation and management of its habitat where the species can be effectively protected (Nash and Johnson, 2014). Regular monitoring of species of conservation interest is essential to plan conservation actions and act according to any changes (Wheatley, 2018). Moreover, conservation of a rare species not only requires an understanding of its ecology, but also the ecology of the species it co-depends on. In this context, larval host plant (LHP) and insect interactions have been a subject of intensive study, especially when either/or both species are in decline (Brunbjerg *et al*., 2017). An example of insects that usually depend significantly on one or several LHPs for larval development are butterflies and moths (order Lepidoptera, L. 1758) and their conservation therefore largely depends on protecting and managing habitats that contain these host plants (Bubova *et al*., 2015). Understanding the relationship and factors determining the survival and distribution of LHPs is key in aiding the conservation efforts for many of these species, where some rely on only a handful of plant species for reproduction and/or food, such as the glanville fritillary *Melitaea cinxia* with *Plantago lanceolata* and *Veronica spicata* (Hanski and Singer, 2001); the white-spotted sable *Anania funebris* and *Solidago virgaurea* (Parsons, 1993); the scarce fritillary *Euphydryas maturna* and *Fraxinus excelsior* (Lindman *et al*., 2018) and the marsh fritillary *Euphydryas aurinia* and *Succisa pratensis* (Brunbjerg *et al*., 2017).

In Europe, many specialist butterfly species who rely on only one or a small number of LHPs have declined due to habitat loss and/or changes in management practices of habitats containing LHPs (Hodgson *et al*., 2009). In some cases, only small and isolated populations of the plants and the associated butterfly species remain (Fox *et al*., 2015). These fragmented remnants are usually where conservation efforts are most needed to support populations through prescriptive measures informed by research into the LHPs and the species they serve, such as in the case of orpine *Sedum telephium* and the apollo butterfly *Parnassius apollo* (Brommer and Fred, 1999) and the marsh fritillary *Euphydryas aurinia* which utilises devil's bit scabious *Succisa pratensis* as its LHP (Brunbjerg *et al*., 2017). In order to better understand the requirements of LHPs and the larvae of butterfly species that subsist on them and therefore identify priorities for conservation efforts, information is required on the origins, resource requirements, reproduction, interactions with other species and the environmental conditions LHPs favour (Begon *et al*., 2006).

For example, in Denmark, the marsh fritillary suffered a population collapse due to a lack of abundance of the LHP (Brunbjerg *et al*., 2017). Researchers investigating this collapse suggested that conservation managers need to take LHP habitat requirements into account, when conserving declining invertebrates. LHPs that occur in high density over a large area are more likely to sustain populations of rare invertebrates that feed on them (Warren, 1987c).

In the coppiced woodlands of southern England, the pre-diapause larvae of the heath fritillary *Melitaea athalia* R. 1775 (Lepidoptera: Nymphalidae) utilise common cow-wheat *Melampyrum pratense* L. 1753 (Lamiales: Orobanchaceae), abbreviated as CCW from hereon, as its primary LHP (Warren, 1987a). CCW is locally common in the UK (Cheffings and Farrell, 2005), but according to The Butterfly Red List for Great Britain, the heath fritillary is listed as endangered. This is due to a decline in occupancy, existing in fragmented populations, and experiencing dramatic fluctuations in abundance, despite being categorised as Least Concern by the International Union for Conservation of Nature (IUCN) in continental Europe (Fox *et al*., 2010). It is also a UK Biodiversity Action Plan (UKBAP) species (Holloway *et al*., 2003) and protected under schedule 5 of the Wildlife and Countryside Act 1981 (Warren, 1985). It is restricted to areas in Kent (The Blean Woodland complex), Essex, Somerset, and Devon (including Exmoor) and Cornwall.

Warren and Key (1991) encouraged more research into woodland invertebrates such as the heath fritillary including habitat requirements and life cycle ecology. One example where this is evident is with the heath fritillary and CCW, which have been the subject of previous research (Hodgson *et al*., 2009; Holloway *et al*., 2003; Masselink, 1980; Salonen *et al*., 2000; Warren, 1985, 1987a, b, c; Winkler & Heinken, 2007), with most studies of these species in the UK having been carried out in the Blean Woodland complex in South-East England, due to the apparently stable and comparatively large population of heath fritillary there compared to other locations in Essex and the South-West. However, local distribution patterns and factors affecting the presence of CCW are not yet fully understood and remain a concern for planning and executing management plans for the conservation of the heath fritillary.

1.1 The heath fritillary *Melitaea athalia*

The heath fritillary (Figure 1) is a generally univoltine species that occurs in open areas including grassland, heathland, and woodland (glades, rides and coppice plots) that it utilises during its short flight season (late May to July). The adult's life span ranges from five to ten days (Warren, 1985). Ovipositing females use both the LHPs and nearby species, with egg batches ranging from 15 to 150 (Warren, 1987a).

Pre-diapause larvae of the 6^{th} instar (Figure 2) emerge from hibernation in late March during warm, sunny weather requiring enough newly emerged CCW shoots in woodland and heathland habitats to feed on as the primary LHP. Larvae are also known to use ribwort plantain *Plantago lanceolata* and germander speedwell *Veronica chamaedrys* as secondary LHPs (Warren, 1987a).

Figure 1. Heath fritillary *Melitaea athalia* (Source: Harris, 2018)

Due to the limited ability of heath fritillary adults to disperse post emergence (typically <200m from the point of emergence) and their sedentary nature, adults need new or established open habitat close-by to forage in and reproduce. Therefore, rotational coppicing in woodland habitats has been successful because it creates new lighter areas with a more open canopy (Hodgson *et al*., 2009). Any new habitats need to be readily accessible and within 300m from an existing colony for heath fritillary adults to be able to reach and then colonise them (Warren, 1987b). New colonies within newly created coppice plots tend to reach their peak in the second and third years, before declining following succession that results in a shadier environment as a result of the development of a dense shrub layer (Bourn *et al*., 2012).

Figure 2. Heath fritillary pre-diapause larvae of the 6th instar (Source: UK Butterflies, 2018)

Heath fritillary metapopulations are generally small (10-100 individuals) to medium (100-250 individuals) in size but given an optimal stable habitat in which to reproduce, large numbers (>250 individuals) can occur in one location (Warren, 1987a). Such larger populations can also act as a reserve from which new satellite populations can develop (Warren, 1987b). Current management regimes aimed at increasing heath fritillary populations include rotational coppicing (Warren and Key, 1991), which has been well implemented, together with creating ecological corridors (inter-connected rides or paths) where the heath fritillary can migrate along and move into new areas (Wheatley, 2018). These types of open habitats are also thought to encourage CCW in deciduous woodland (Holmes and Wheaten, 2002).

1.2 Common cow-wheat *Melampyrum pratense*

CCW is an annual herbaceous plant species flowering from late spring to late summer (Figure 3). According to the Red Data List of Vascular Plants of Great Britain, CCW is categorized to be of Least Concern (Cheffings and Farrell, 2005). It is a hemiparasitic plant, which means that it usually depends on both herbaceous and woody host plants for at least some of its nutrition (Winkler and Heinken, 2007). CCW achieves this by tapping into the roots of adjacent plants such as *Quercus sp.*, *Betula sp.* and *Vaccinium myrtillus* by haustoria (Salonen *et al*., 2000). CCW is acidophyilic, preferring moderately acidic conditions between pH 4-5 and requires sufficient sunlight (semi-shade) and adequate levels of soil calcium, whereas it does not appear to favour areas with high amounts of shrub cover, organic matter, canopy cover, nitrogen and potassium (Humpage, 2004).

Habitats where CCW can occur include both deciduous and coniferous woodland (Salonen *et al*., 2000; Winkler and Heinken, 2007), scrub, heaths, and moorlands up to 960 metres elevation (NBN Atlas, 2018) and where suitable host species exist (Svetlikova *et al*., 2018). It can also be found distributed amongst grasses including wavy hair-grass *Deschampsia flexuosa* (Masselink, 1980). In the south-east of England, it is an ancient woodland indicator that typically occurs where rotational coppicing and the autumnal mowing of rides and glades are part of the management practices (Holmes and Wheaten, 2002).

Figure 3. Common cow-wheat *Melampyrum pratense* (Source: Harris, 2018)

Individual plants produce an average of 60 seeds that fall from July to September (Masselink, 1980). Seeds are typically dispersed by barochory (dispersal by gravity) and myrmecochory (dispersal by ants). CCW seeds are reliant on these dispersal mechanisms as they have no features that facilitate wind dispersal (Winkler and Heinken, 2007). Due to the plant's height (max. 50cm), the seeds usually fall close to the parent plant. CCW seed germination occurs at low temperatures in the autumn, before seedlings emerge in late March (Winkler and Heinken, 2007).

Seed survival rates for CCW tend to be higher in dense grassy locations but are dependent on the occurrence of seed eating animals (Masselink, 1980). However, low germination rates can occur in dense grass tufts, where myrmecochory (dispersal by ants) may increase survival rates of seeds due to them being transported to more suitable locations (Masselink, 1980). The Blean Woods NNR accommodates the wood ant *Formica rufa* L. 1761 (Hymenoptera: Formicidae) (Welch, 1978; pers. obs., 2018) which can disperse the seeds of CCW. Ants are attracted by a fatty substance within the elaiosome on the seeds (Masselink, 1980).

Mycorrhizal fungal associations can be of importance for CCW, considering the nutrient poor environments where it typically occurs, where mycorrhizal fungi can compensate for poor soil conditions by providing nutrients to the host plant (Salonen *et al*., 2000). It has been suggested that the biomass of CCW growing with a mycorrhizal host can be higher than that of the plants attached to non-mycorrhizal CCW and can produce three times more flowers. However, this is to the detriment of the host species with which CCW has a hemiparasitic relationship (Salonen *et al*., 2000).

1.3 The Blean Woods National Nature Reserve

The Blean Woods National Nature Reserve (NNR) is owned by a Partnership including Natural England, the Royal Society for the Protection of Birds (RSPB), the Woodland Trust and the three councils of Canterbury City, Kent County and Swale Borough, but is managed by the RSPB (Nash and Johnson, 2014). This ancient woodland thought to exist before 1600 has complex management requirements due to the existence of several important species, including the heath fritillary, which all need both short and long-term planning from informed research (Fuller and Peterken, 1995). In the Blean NNR, rotational coppicing and the autumnal mowing of glades and rides with existing heath fritillary metapopulations are the current management practices to maintain and increase colonies (Nash and Johnson, 2014). These practices have seen colonies increase in the years from 2004 to 2011 (Bourn *et al*., 2012). Both in the NNR and in the wider Blean complex the traditional woodland management technique of coppicing has been implemented as one of the main management regimes and carried out by conservation organisations and private landowners (Holmes and Wheaten, 2002). Coppicing stems back some 6000 years and became a dominant medieval practice until its decline in the last century (Warren and Key, 1991). The nature of rotational coppicing, creates open areas where coppice stools, shrub and ground cover increase steadily if left unchecked as succession takes place, resulting in competition for light and nutrients (Fuller and Peterken, 1995). It is widely thought that woodland species such as the heath fritillary have adapted to the conditions coppicing provides and due to their dependence on areas of open canopy they have declined as a result of the decline in woodland coppicing (Hodgson *et al*., 2009). This management regime is also beneficial to other species, such as the nightingale *Luscinia megarhynchos* B. 1831, and is ecologically important since deer are currently absent from the whole of the wider Blean complex (Bourn *et al*., 2012). Current woodland management therefore often includes elements of coppicing, thinning, and mowing of glades and rides to preserve or create areas of open canopy (Molder *et al*., 2019).

1.4 CCW distribution in the Blean Woods NNR

Distribution surveys of CCW in the Blean have been carried out by both the RSPB and Butterfly Conservation. The RSPB surveyed CCW as part of their management plan objectives in 2008 and 2013 at the Blean Woods NNR (Nash and Johnson, 2014), whilst in 2018 Butterfly Conservation surveyed CCW in the entire Blean Woodland complex (Wheatley, 2018).

The RSPB distribution surveys revealed changes over a five-year period showing a decline of CCW locally in areas where succession had taken place but increases in others that had been recently coppiced (Nash and Johnson, 2014). On a larger scale CCW distribution did not change dramatically across the Blean Woods NNR with the 2018 Butterfly Conservation survey revealing a similar distribution pattern to that reported in the 2013 survey (Wheatley, 2018).

As it can be seen from both the RSPB (2008, 2013) and Butterfly Conservation (2018) surveys, the current conservation managers implement regular monitoring of CCW by surveying planned routes at appropriate times of the year to inform decisions on the conservation management for the heath fritillary. In addition, they also require knowledge of factors driving the presence and abundance of the heath fritillary Such information is also important for other areas where the heath fritillary occurs and is the subject of conservation efforts, for example in the wider Blean complex and in Essex, where similar habitats exist (Perry, 2009).

Only two studies have investigated the ecology of CCW on a local scale in the Blean Woods NNR (Humpage, 2004; Underdown, 1995). Underdown (1995) primarily investigated the effects of ground cover on CCW, as it was thought that as a hemiparasitic species, CCW would be more abundant in vegetated areas rather than areas with bare ground. A secondary aspect of this study was to find associations with floral species that might be potential hosts for CCW. There was a significant positive relationship with tree cover and negative relationships with bramble *Rubus fruticosus* and great woodrush *Luzula sylvatica* (Underdown, 1995). It is unclear what the variable tree cover represented, but it has been interpreted as the quantity of trees in a survey area, not canopy cover, which could infer a hemiparasitic relationship between CCW and the surrounding trees. The amount of bare ground in each area could be an important variable affecting CCW, as a lack of other plant species could determine the availability of potential host species that in turn affect CCW distribution and survival (Masselink, 1980). Warren (1987a) discusses how bare ground and sparse CCW can negatively affect the development of heath fritillary larvae due to a lack of available food and an increased predation risk.

Humpage's study (2004) included additional variables thought to affect CCW in a further attempt to understand the plant's ecology and possibly reveal which variables had the most significant effect on CCW local presence, absence, and frequency. Bare ground was included as one of the variables studied, together with ground layer cover (individual plant species), shrub layer cover, canopy cover, soil composition, soil pH and light intensity.

Significant relationships were found between CCW presence and absence in surveyed quadrats and the canopy cover, ground layer cover, shrub layer cover, soil calcium content and soil pH. Soil calcium (Ca) content and soil pH are known to be important factors for plant nutrition that therefore typically also drive plant distribution (Rengel and Elliot, 1991). Ca availability is at its lowest level when acidity is strong to moderate, around pH 4-5, but is at its highest level in neutral to alkaline conditions of pH 7-9 (Vincent, 1990). Ca uptake in plants can be restricted by increased aluminium (Al) in acidic environments (Rengel and Elliot, 1991). Therefore, the distribution of acidophilic species such as CCW may be more sensitive to Ca concentrations and Ca availability in the soil. Ca and pH and their interplay could therefore contribute to CCW abundance. Humpage (2004) found that CCW was absent when soil pH was below 4.3, which infers that CCW is sensitive to small changes in acidity. These changes can be influenced by a range of factors, including biotic ones. Ants have been shown to alter soil pH, with changes found mostly on the periphery of their nests, possibly by the accumulation of organic matter affecting soil chemistry (Frouz and Jilkova, 2008). Therefore, the distance from an ant nest could have an influence on soil pH and by extension on CCW abundance. Proximity to an ant nest can have additional effects depending on the species, such as ants facilitating seed dispersal (Winkler & Heinken, 2007).

As a hemiparasitic plant species that is likely to parasitise the roots of neighbouring plants in the soil, understanding CCW ecology should include an investigation of other plant species above ground that may act as its hosts or otherwise influence its nutrition (Masselink, 1980; Salonen *et al*., 2000; Underdown, 1995). Hemiparasitism interactions can be triggered by an autonomous environmental response or induced by chemical signals from the host plant to a seed (Tesitel *et al*., 2015). It is believed species in the genus *Melampyrum*, such as CCW germinate autonomously from environmental cues including temperature and then seek out a suitable host to extract nutrients (Tesitel *et al*., 2015). Oak *Quercus sp.* and their saplings could be act as hosts from which CCW extracts important nutrients (Salonen *et al*., 2000) and was one of the species assessed by Humpage (2004). However, this specialism and adaptation to specific hosts can impose constraints on CCW to form a host connection and produce a viable population (Tesitel *et al*., 2015).

The quantity of light available on a woodland floor can be an important factor affecting a plant species' distribution and abundance (Messier *et al.*, 1998; Valladares *et al*., 2016**)**. Light availability is most directly influenced by the amount of canopy cover preventing direct sunlight from reaching the woodland floor (Valladares *et al*., 2016). Under heavily shaded conditions only shade-tolerant species can subsist, whilst some species are adapted to semi-shade and/or full direct sunlight (Begon *et al*., 2006). CCW is a semi-shade species and its local presence seems to be significantly related to the amount of canopy cover (Humpage, 2004).

1.5 Aims

Local distribution patterns and factors affecting CCW in the Blean Woods NNR are not yet fully understood. To inform and support current management efforts to encourage CCW and thereby the heath fritillary, the current study aims to investigate what variables if any, affect local CCW distribution and abundance and if there are patterns in its distribution in the Blean Woods NNR. It is expected such research will also be beneficial to those managing habitats accommodating the heath fritillary in the wider Blean complex and possibly in Essex where similar habitats exist.

To examine local CCW distribution in the Blean Woods NNR, % cover data for CCW in thirty $100m^2$ grid squares was gathered and related to biotic and abiotic environmental variables to address the following research questions: -

a) Which environmental variable(s) significantly predicts CCW cover?

b) Is CCW distribution at a local scale ($<100m^2$) random or clumped?

c) Does CCW reoccur in the same areas over time?

2. METHODS

2.1 *Study area*

Blean Woods National Nature Reserve (NNR) in Kent (51°30’ N, 01°01’ E) is situated north-west of the city of Canterbury (Figure 4), between the Thames estuary to the north and the Stour valley to the south (Holmes and Wheaten, 2002). It is located on the acidic London Clay (Burnham and McRae, 1978) and is an area of ancient woodland that contains the UKBAP species *Melitaea athalia* (Hodgson *et al*., 2009), the heath fritillary, and its larval foodplant common cow-wheat *Melampyrum pretense* (Holmes and Wheaten, 2002).

Figure 4. Blean Woods NNR location in South-east England (Source: Ordnance Survey, 2019, annotated in Digimap).

Blean Woods NNR is only one part (500 ha) of a larger network of continuous ancient woodland in East Kent (Figure 5). This site is designated as a Site of Special Scientific Interest (SSSI) and 50% of its area is also a Special Area for Conservation (SAC) (Nash and Johnson, 2014).

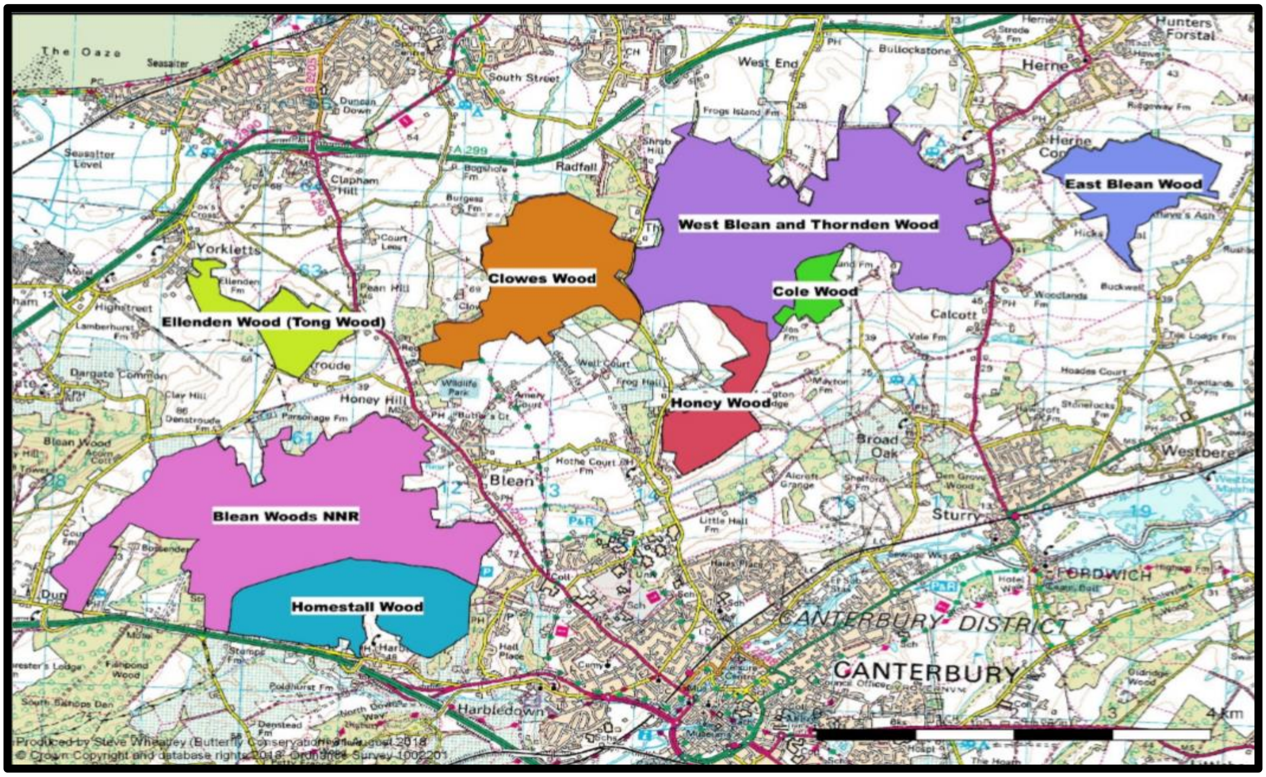

Figure 5. Blean Woods NNR location and the wider Blean Woodland Complex (Source: Wheatley, 2018)

2.2 *Field survey*

Sixty field surveys were carried out from May through to October 2018 to record eight variables in $100m^2$ grid squares. These variables included distance to oak trees, distance to ant nests, light intensity, canopy cover, bare ground cover, soil pH, soil calcium content and oak sapling distribution (Table 1).

Table 1. Predictor variables used to test CCW distribution and abundance

Variable	*Source*
Canopy cover	Humpage, 2004; Messier *et al*., 1998; Underdown, 1995
Bare ground cover	Humpage, 2004; Underdown, 1995
Soil pH	Frouz and Zilkova, 2008; Rengel and Elliot, 1991
Soil Ca content	Humpage, 2004; Rengel and Elliot, 1991
Distance to oak trees	Salonen *et al*., 2000; Masselink, 1980
Distance to ant nests	Frouz and Zilkova, 2008; Winkler and Heinken, 2007
Light intensity	Humpage, 2004; Messier *et al*., 1998; Underdown, 1995
Oak sapling distribution and abundance	Salonen *et al*., 2000; Masselink, 1980

A grid with 10m x 10m (100m^2) dimensions per grid square (Akasaka *et al*., 2005) was overlaid on a map covering the entirety of Blean Woods NNR using QGIS 3. A stratified sampling strategy was implemented to sample grid squares for CCW % cover and the predictor variables. Thirty of the 10m x 10m grid squares within the Blean Woods NNR (Figure 6) were selected from the grid based on historical distribution records of CCW from 2008 and 2013, using the RSPB distribution category GIS map (Figure 7). Twenty-eight grid squares were selected with historical records of CCW at different levels of abundance and two with no historical records of CCW. For example, in grid square 2 (Figure 6) CCW has historically been common, as shown by a red shade (Figure 7). However, in grid square 31 (Figure 6) CCW has not been common historically (Figure 7), with little or none recorded. In addition, the thirty grid squares were selected to include a range of potential CCW habitats present in the NNR including glades (5), rides (13) and paths (12). Grid squares selected were at least 100m distant from each other to maintain spatial uniqueness. Stratified sampling was deemed as appropriate because CCW abundance can be divided into subgroups (strata) using Warren's (1985) LHP abundance scoring method. This sampling method would allow for the sampled grid squares to represent the full range of CCW % cover and potential habitats in different parts of the Blean Woods NNR. In addition, areas historically containing CCW could be focused on at various abundance levels. Random sampling and to a lesser extent, systematic sampling could have resulted in many grid squares containing no CCW, such as in areas of dense woodland, thus leading to many 0 values in the data set.

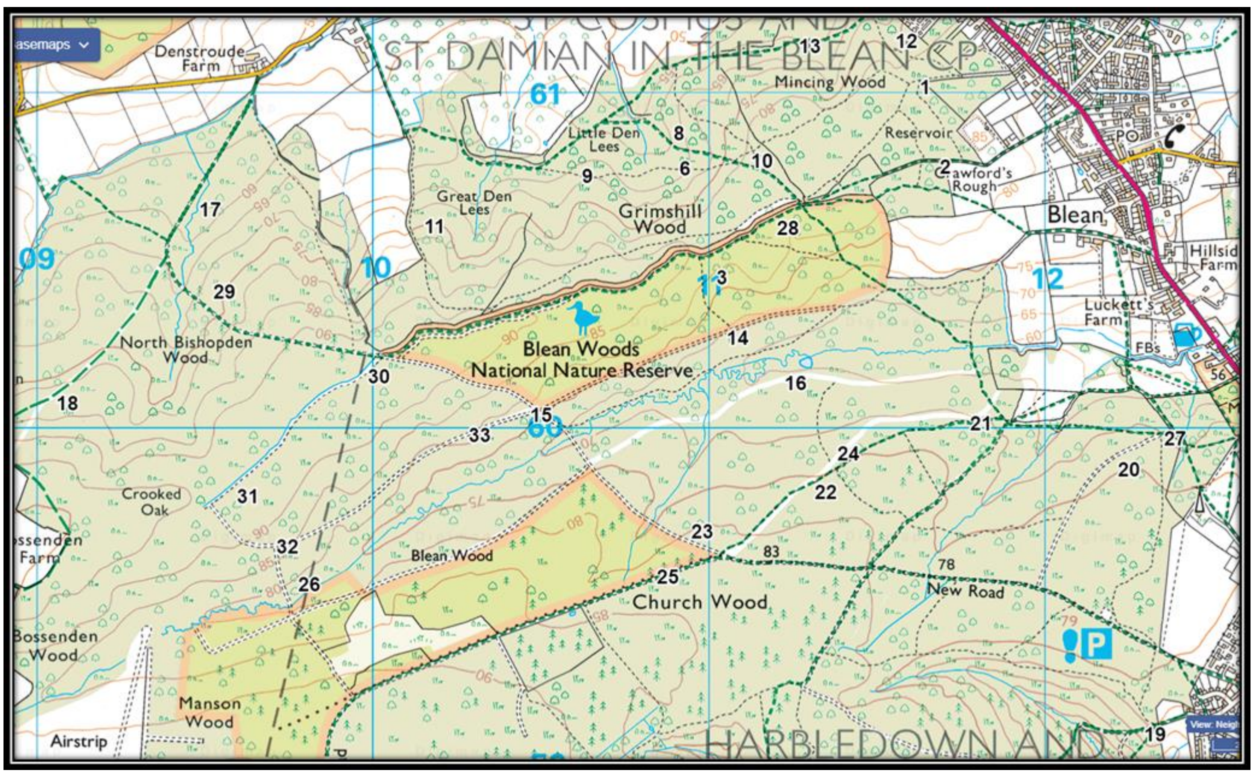

Figure 6. Map of Blean Woods NNR, showing survey locations (Produced using Digimap)

Each $100m^2$ grid square was further subdivided into four hundred 50cm x 50cm ($0.25m^2$) quadrats that were used to record CCW cover and oak sapling cover (Fehmi and Bartolome, 2001; Perry, 2009; Underdown, 1995; Warren, 1985). Thus, each quadrat represented 0.25% of the grid square containing it. If a quadrat contained at least one CCW individual, this was recorded as 0.25% cover for the overall grid square surveyed. An abundance score (Warren, 1985) and a distribution category (Nash and Johnson, 2014) corresponding to those used in previous surveys was used as well as % cover for CCW to allow comparison across surveys.

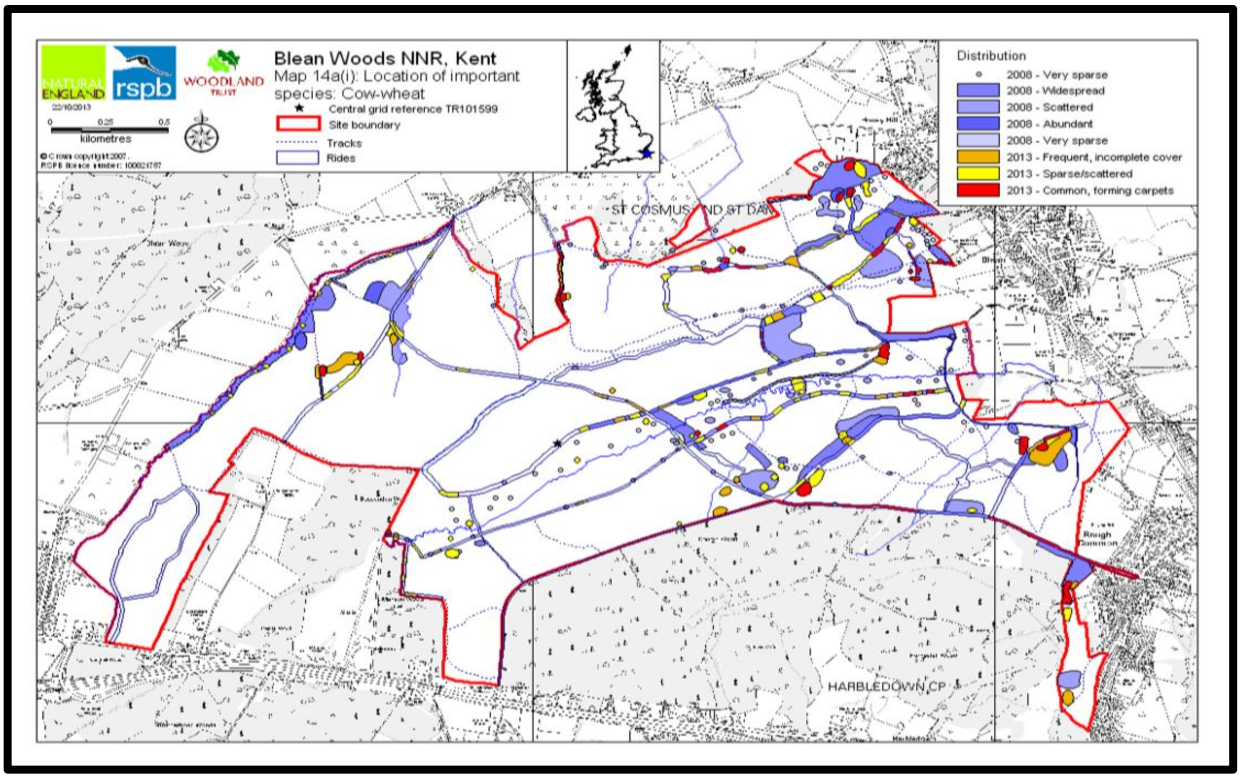

Figure 7. Historical RSPB distribution records of CCW from 2008 and 2013 (Source: Nash and Johnson, 2014)

Warren's (1985) LHP abundance scoring method for CCW (Table 2.) is on a subjective scale of rank abundance ranging from 0-5 (0 = absent to 5 = >40% cover). This method is preferable in survey areas that are $>100m^2$ and was originally implemented as a measure of CCW whilst walking a transect. The RSPB distribution category method (Table 3.) was used to assess CCW as it had been previously in the RSPB 2008 and 2013 surveys.

Canopy cover for each grid square was recorded by standing at the centre of a grid square and estimating the % of the field of view with canopy when looking directly upward. Light intensity was recorded only during clear sky conditions using a Lutron lux meter LX-101 and by holding the device level and 50cm above the height of the ground. Bare ground % cover was estimated in each quarter of a grid square (25m^2) and a mean value was calculated from the four values per grid square. Oak sapling % cover was recorded using the same method to record CCW % cover. Distances to nearest mature oak (> 2m in height) and to the nearest wood ant nest were measured in metres from the centre of each grid square using a tape measure.

Table 2. CCW abundance scores (after Warren, 1985)

Category	*Score*
Absent	0
Rare – a few spikes only <0.1%	1
Scarce – a few patches present 0.1-1%	2
Frequent – patches always in view 1-10%	3
Common – ground cover 10-40%	4
Abundant – ground cover >40%	5

Table 3. RSPB CCW distribution categories (after Nash and Johnson, 2014)

Category	*Abbreviation*
Sparse/scattered	(S/S)
Frequent, incomplete cover	(FIC)
Common, forming carpets	(CFC)

2.3 *Soil sampling and analysis*

Soil samples for laboratory testing of soil pH and calcium content were collected in October 2018. Samples were taken to a depth of 5cm using a soil corer at the centre of each quarter of a grid square, these were then combined into one plastic bag per grid square and mixed. The samples were then air-dried for one week before being oven-dried in foil for 8 hours using a Carbolite Gero AX Oven at 60°C to remove residual water.

Each dried soil sample was then ground into powder using a pestle and mortar before being sieved through a 2000 microns mesh sieve to a final mass of 5g per sample. Soil pH was determined by adding de-ionised water to the filtered soil samples and mixing them for one hour using a Stuart Orbital Incubator SI500 set at 120 rpm. Soil pH was then determined by inserting the probe of a Jenway pH meter 3510 into the solution once mixing was complete. Calcium content of soil was measured by first digesting soil samples using HNO_3 (>68%) and HCl (37%) in the Berghof Speedwave 4 Microwave Digester following the procedure DIN EN 13346. 2.5 ml of HNO_3 and 7.5 ml HCl were added to 0.75g of dried soil sample. After microwave digestion the samples were filtered using Whatman 70mm filter paper and then made up to 100ml with de-ionised water. Following this, an ICP-OES Optima 8000 PerkinElmer was used to measure Ca content (mg/L) against a standard. Standards were then made up to the same volume using a calcium standard for ICP (19051-100ML-F), HNO_3 (>68%) and HCl (37%) at 5, 10 and 20 ppm with a blank solution.

2.4 *Data Analysis*

Two methods were used to reduce the number of independent variables potentially affecting CCW % cover in grid squares prior to performing linear regression analysis: First, independent variables were correlated against each other using Spearman's rho (not all independent variables were normally distributed; Anderson-Darling test, $\alpha=0.05$) to identify collinearity between them.

Second, a principal component analysis (PCA) was used to reduce the independent variables into values of linearly uncorrelated variables (Karamizadeh *et al*., 2013). The main reasoning for using a PCA was to reduce the number of variables into a smaller number of derived variables that could be plotted in 2-dimensional space and be subjected to further analysis (Quinn and Keough, 2002). The following eight variables used in the PCA: - canopy % cover, light intensity, bare ground % cover, oak sapling % cover, distance to nearest mature oak and ant nest, soil pH and soil Ca content.

A correlation matrix was used in the PCA as the variables were measured differently (% cover, length, scale, and concentration). The scree plot showed a moderate break between principal components two and three and these two components were chosen for regression against the response variable (CCW % cover). The first four principal components had eigenvalues >1 and together accounted for 73.1% of the variation in the independent variables, though there was no distinctive break in the scree plot. A multiple regression model was fit to the first four principal components as independent variables with CCW % cover as the dependent variable. A loading plot was generated for the first two principal components to identify the independent variables contributing most to the variation captured by them.

First, a stepwise regression model was used with backward elimination of terms (α to remove predictor = 0.1) to relate CCW % cover to other measured variables and incorporated all eight variables. Second, a multiple linear regression model was used with three independent variables selected following the test correlation tests for collinearity. The independent variables selected were canopy cover (%), oak sapling cover (%) and distance to nearest ant nest (m).

In all regression models (multiple regression, stepwise regression and principal component regression), the residuals of the models were initially not normally distributed (Anderson-Darling test, α=0.05), so Grubbs' test for outliers was used on the residuals of the models. The residuals for grid square 11 were identified as a significant outlier in each regression ($P<0.001$) and therefore removed from linear regression analysis. This resulted in normally distributed residuals.

The randomness of CCW distribution within each quadrat was tested by subdividing each square into 16 portions of equal size (each containing 5*5=25 quadrats) and testing the number of quadrats with CCW in each portion against the expected number predicted by randomness according to a Poisson-distribution. Expected numbers were calculated using the equation for the Poisson-distribution

$$f(x) = \frac{m^x}{x!} e^{-m}$$

where *m* is the mean number of quadrats with CCW in a quadrat and *x* is the count of events under observation. Observed and expected numbers of grid square portions containing CCW were then compared using a Chi-Squared Goodness-of-Fit test. To minimize the number of cells in each test, categories were combined until all but at most one of the cells containing observed numbers were ≥5. This typically results in tables with three categories. This analysis was not possible for two grid squares that contained no CCW (30 and 31).

Median CCW % cover was compared between habitat types (glade, path and ride) and by classification of grid squares according to CCW survey data for 2013 using Kruskal Wallis tests (residuals for General Linear models were not normal before and after arcsin-transformation of CCW % cover data).

The significance level for all tests was $P < 0.05$.

All statistical analyses were carried out using Minitab v17 (MiniTab, 2019).

3. Results

3.1 *CCW abundance*

CCW % cover ranged from 0% to 72.75% across the 30 grid squares surveyed (Table 4). The mean % cover was 14.5% (standard deviation: +/- 2.3%). CCW abundance scores (after Warren, 1985) revealed that most of the grid squares surveyed fell into the categories of 'abundant' or 'frequent' categories (Table 5). CCW distribution categories (after Nash and Johnson, 2014) showed that most of the grid squares fell in the frequent, incomplete cover (FIC) category (Table 6). Due to using a stratified sampling method these results only reveal the abundance and distribution of CCW within the 30 surveyed grid squares and do not represent the whole Blean Woods NNR.

CCW was found in grid squares mainly containing a soil pH value of between 4-5. Soil pH ranged from 3.7 to 5.3 with a mean of 4.4. Four grid squares had a soil pH of <4 and three grid squares had a soil pH of >5.

Table 4. Grid square location with % cover of CCW with abundance and distribution

Location	*CCW % Cover (m^2)*	*Abundance*	*Distribution*
TR 11588 61024 (1)	12.75	4	FIC
TR 11647 60797 (2)	13.75	4	FIC
TR 10981 60461 (3)	22	4	FIC
TR 10870 60788 (6)	15.75	4	FIC
TR 10855 60904 (8)	5.25	3	FIC
TR 10592 60759 (9)	27.5	4	FIC
TR 11090 60825 (10)	16.5	4	FIC
TR 10112 60605 (11)	72.75	5	CFC
TR 11519 61166 (12)	8.75	3	FIC
TR 11228 61153 (13)	3.5	3	FIC
TR 11014 60274 (14)	1.75	2	S/S
TR 10429 60043 (15)	4.25	2	S/S
TR 11182 60148 (16)	9.5	4	FIC
TR 09423 60629 (17)	7.75	3	FIC
TR 09021 60081 (18)	35	4	CFC
TR 12253 59101 (19)	22.25	4	CFC
TR 12214 59925 (20)	9.75	3	FIC
TR 10871 59705 (21)	10.5	4	FIC
TR 11188 59747 (22)	11	4	CFC
TR 11733 60040 (23)	4.75	3	FIC
TR 11326 59957 (24)	3.75	2	S/S
TR 10821 59560 (25)	8.75	2	S/S
TR 09741 59536 (26)	11.75	4	FIC
TR 12305 59976 (27)	27	4	CFC
TR 11159 60603 (28)	63.5	5	CFC
TR 09462 60400 (29)	3	2	S/S
TR 09954 60164 (30)	0	0	/
TR 09545 59806 (31)	0	0	/
TR 09668 59657 (32)	0.75	1	S/S
TR 10239 59989 (33)	0.75	1	S/S

Table 5. CCW abundance scores with quantity of grid squares (after Warren, 1985)

Category	*Score*	*Quantity of grid squares*
Absent	0	2
Rare – a few spikes only <0.1%	1	2
Scarce – a few patches present 0.1-1%	2	5
Frequent – patches always in view 1-10%	3	6
Common – ground cover 10-40%	4	13
Abundant – ground cover >40%	5	2
Total grid squares		<u>30</u>

Table 6. RSPB CCW distribution categories with quantity of grid squares (after Nash and Johnson, 2014)

Category	*Abbreviation*	*Quantity of grid squares*
Sparse/scattered	(S/S)	7
Frequent, incomplete cover	(FIC)	15
Common, forming carpets	(CFC)	6
Total grid squares containing CCW		<u>28</u>

3.2 *Relationship between CCW abundance and measured variables*

3.2.1 Collinearity of independent variables

When all independent variables were correlated with each other to check for collinearity, significant correlations were found for distance to nearest oak and canopy cover, soil calcium content and oak sapling cover, soil pH and distance to nearest ant nest and the two soil parameters (pH and calcium content) (Table 7).

Table 7. Cross correlation (Spearman's rho) of all predictor variables considered for inclusion multiple regression model. Values in italics are values for Spearman's rho, followed by corresponding P-value. Significant correlations are bold. CC = canopy cover, LI = light intensity, BGC = bare ground cover, OSC = oak sapling cover, DNO = distance to nearest oak, DNA = distance to nearest ant nest, pH = soil pH, Ca = soil calcium content

	CC	LI	BGC	OSC	DNO	DNA	pH	Ca
CC		*-0.359* 0.051	*0.063* 0.742	*0.156* 0.412	***-0.564*** **0.001**	*0.164* 0.387	*-0.094* 0.621	*0.074* 0.697
LI			*0.178* 0.347	*-0.288* 0.122	*0.065* 0.733	*-0.194* 0.305	*0.033* 0.865	*0.194* 0.304
BGC				-0.202 0.285	*0.157* 0.407	*-0.121* 0.525	*0.043* 0.821	*-0.02,* 0.888
OSC					*-0.213* 0.259	*-0.053* 0.780	*-0.161* 0.397	***-0.452*** **0.012**
DNO						*0.061* 0.747	***0.369*** **0.045**	*0.186,* 0.325
DNA							*0.142* 0.454	*0.220* 0.242
pH								***0.609,*** **<0.001**

Based on the results of the check for collinearity, three independent variables were selected for inclusion in a multiple linear regression model: % canopy cover, % cover oak saplings and distance to nearest ant nest (m). None of these variables showed collinearity with each other and represented variables suspected to influence CCW distribution based on the literature (Humpage, 2004).

3.2.2 Multiple and stepwise regression models

In the backwards stepwise regression model (Table 8), the % cover of oak saplings and the soil Ca content had a significantly positive effect on the % cover of CCW (% cover oak saplings: T = 6.43, Coef. = 1.271. P<0.001; Soil Ca: T = 2.95, Coef. =-0.217, P = 0.007). These were also identified as independent variables with collinearity based on correlation of independent variables. The only other variable retained in the final regression model was the distance to the nearest ant nest, though this relationship was not significant (T = 2.01, Coef. =0.192, P = 0.055). In the multiple regression model (Table 9), only % cover of oak saplings significantly predicted % CCW cover (T = 5.08, Coef. = 1.162. P<0.001; R^2 = 0.59).

Table 8. Results of the multiple linear regression of % CCW cover as a dependent variable against the first four principal components generated by the PCA correlation matrix. Adj SS= adjusted sum of squares; Adj MS = adjusted mean of squares

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	3	3400.2	1133.40	18.13	0.000
% cover (m^2) oak saplings	1	2583.6	2583.64	41.34	**0.000**
Distance to nearest ant nest (m)	1	253.4	253.38	4.05	0.055
Soil Ca content (mg/L)	1	544.2	544.24	8.71	0.007
Error	25	1562.5	62.50		
Total	28	4962.7			

Table 9. Multiple regression model results of CCW % cover as a response variable regressed against canopy cover (%), oak sapling cover (%), distance to nearest ant nest (m). (Produced using MiniTab v 17). Adj SS= adjusted sum of squares; Adj MS = adjusted mean of squares

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	3	2920.43	973.48	11.92	<0.001
Canopy Cover (%)	1	64.45	64.45	0.79	0.383
% cover (m^2) oak saplings	1	2110.03	2110.03	25.83	<**0.001**
Distance to nearest ant nest (m)	1	219.89	219.89	2.69	0.113
Error	25	2042.29	81.69		
Total	28	4962.72			

3.2.3 Principal component analysis and regression

The scree plot and correlation matric for the principal component analysis indicated that the first four principal components had eigenvalues >1 and together accounted for 73.5% of the variation in the independent variables, though there was no distinctive break in the scree plot (Figure 8). A multiple regression model was fit to the first four principal components as independent variables with % CCW cover as the dependent variable. The results indicated that only the first two principal components explained a significant amount of variation in % CCW cover (Table 10).

Table 10: Results of the multiple linear regression of % CCW cover as a dependent variable against the first four principal components generated by the PCA correlation matrix

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	4	2008.04	502.1	7.78	<0.001
Principle component 1	1	578.57	578.57	8.97	**0.006**
Principal component 2	1	1280.16	1280.16	19.84	<0.001
Principal component 3	1	45.33	45.33	0.70	0.410
Principal component 4	1	103.97	103.97	1.61	0.216
Error	24	1548.73	64.53		
Total	28	3556.77			

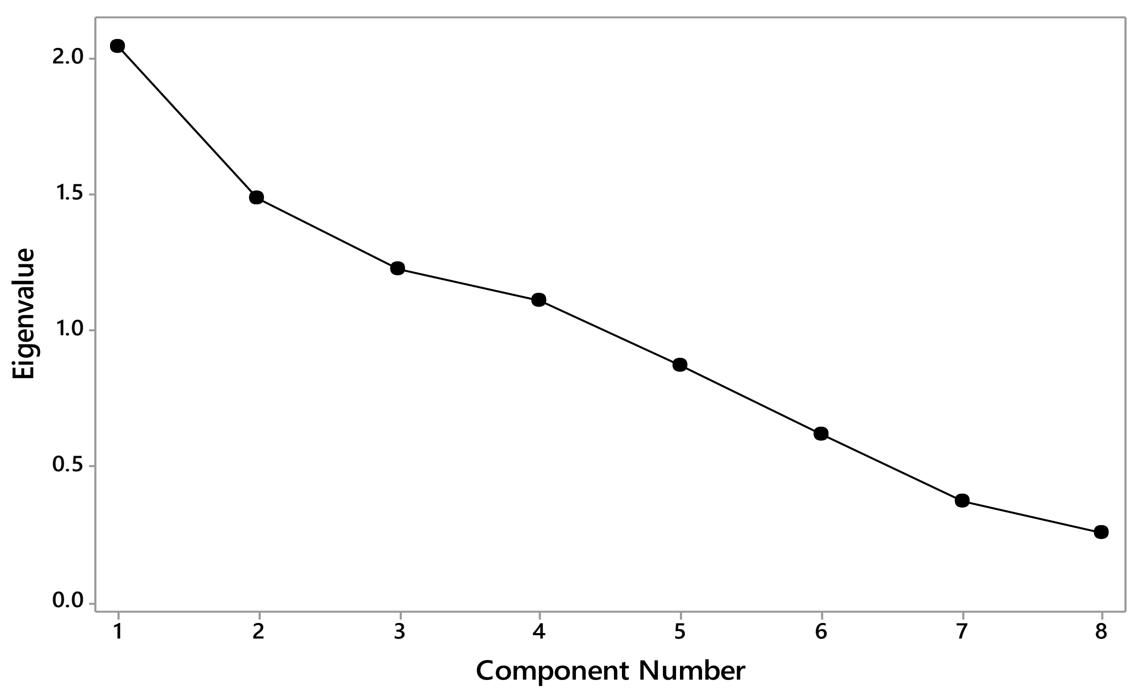

Figure 8. PCA Scree plot showing eigenvalues for the eight principal components generated from the eight independent variables (Produced using MiniTab v 17)

Table 11. Eigenanalysis of the Correlation Matrix for the principal component analysis of the independent variables (Produced using MiniTab v 17)

Principal component	1	2	3	4	5	6	7	8
Eigenvalue	2.0485	1.4918	1.2288	1.1137	0.8721	0.6192	0.3726	0.2533
Proportion	0.256	0.186	0.154	0.139	0.109	0.077	0.047	0.032
Cumulative	0.256	0.443	0.596	0.735	0.844	0.922	0.968	1.000
Variable								
Canopy Cover (%)	-0.416	-0.342	0.508	-0.154	-0.117	0.163	-0.122	-0.611
Light Intensity (lux)	0.325	0.258	0.265	0.495	0.548	-0.157	0.062	-0.429
Bare Ground Cover (%)	0.074	0.294	0.610	-0.447	-0.040	-0.500	0.151	-0.429
% cover (m²) oak saplings	-0.417	-0.260	-0.260	0.266	-0.032	-0.780	0.073	-0.071
Distance to nearest oak (m)	0.428	0.041	-0.397	-0.515	-0.078	-0.254	-0.210	-0.526
Distance to nearest ant nest (m)	-0.135	-0.367	-0.093	-0.403	0.803	0.043	0.039	0.171
Soil Acidity (pH)	0.420	-0.528	0.069	0.029	-0.173	0.016	0.712	-0.046
Soil Ca content (mg/L)	0.405	-0.498	0.247	0.181	-0.057	-0.151	-0.633	0.260

The loading plot revealed that the first principal component was mainly capturing effects of canopy cover, oak sapling cover and distance to the nearest oak (though the latter seemed to affect the first principal component inversely to oak sapling cover). This component could therefore be considered as a factor encompassing mainly oak-associated effects (since oaks also contributed to canopy cover. The second principal component was capturing effects of distance to the nearest ant nest and bare ground cover and could be considered as a factor encompassing other effects (other vegetation, ant nests). Soil properties (calcium content and pH) seemed to contribute strongly to both of the first two principal components.

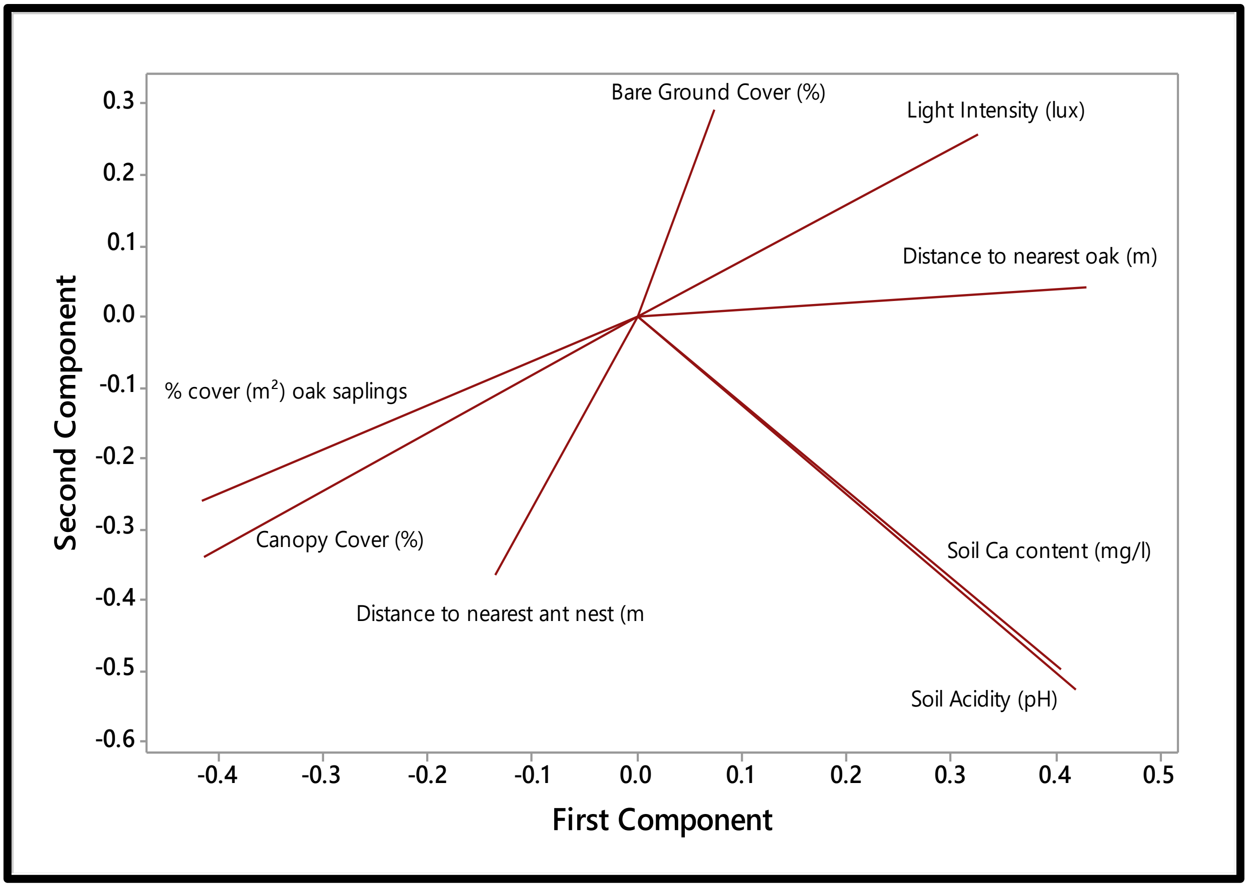

Figure 9. PCA loading plot for the first two principal components of the independent variables

(Produced using MiniTab v 17)

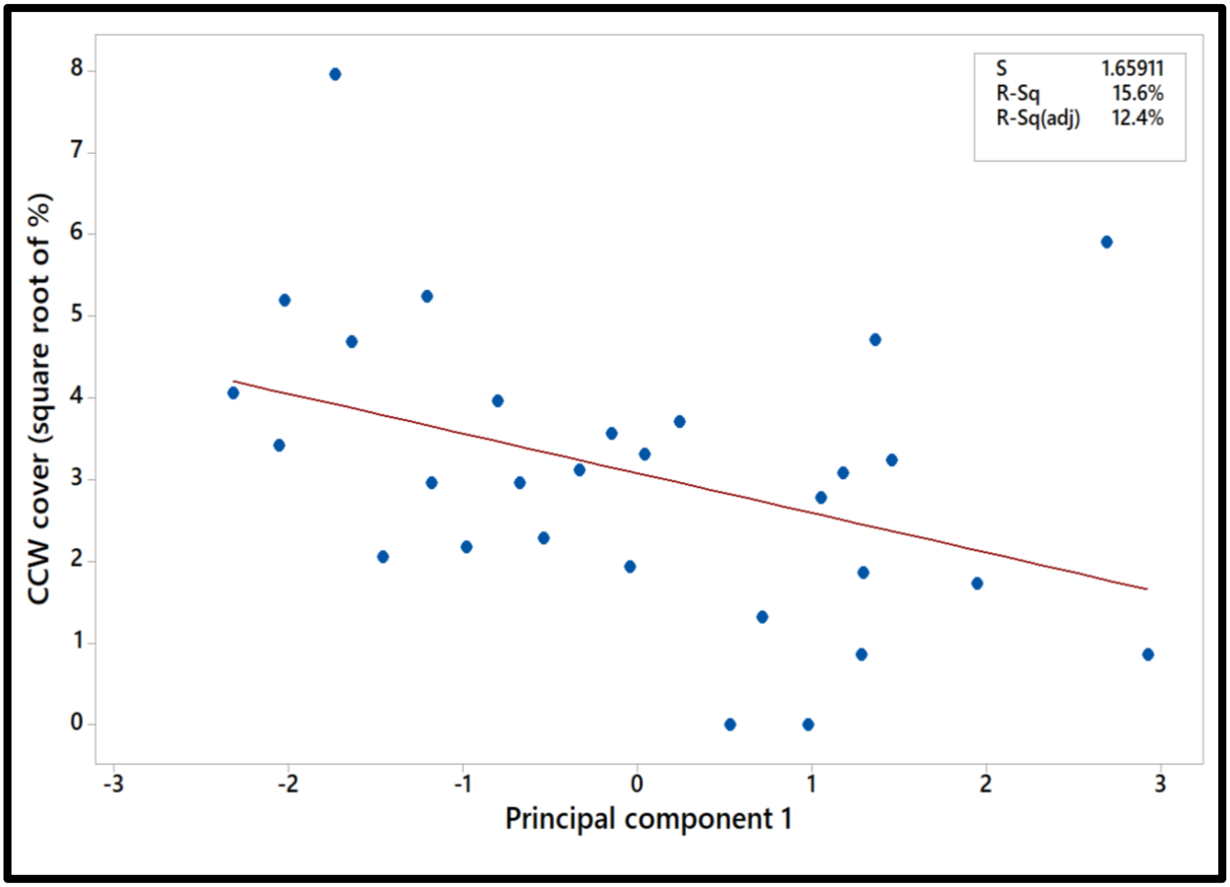

Figure 10. Linear regression of the square root of CCW % cover against the first principal component value from the PCA corresponding to each grid square with fitted trendline

For both principal components one and two, CCW % cover increased with decreasing values of the principle component (Figures 10 and 11). Considering the regression results for the principal components in light of the PCA loading plot (Figure 9) and together with the results of the multiple and stepwise regression models, it appears that oak-associated variables, primarily the presence of oak saplings in grid squares, had the greatest predictive power for of CCW % cover in grid squares. Other factors such as bare ground cover and distance to the nearest ant nest may also contribute, but given the lack of significant relationships of CCW % cover with these variables in the regression models and the contribution of multiple variables to principle component 2, the relative importance and interaction between these independent variables is not clear.

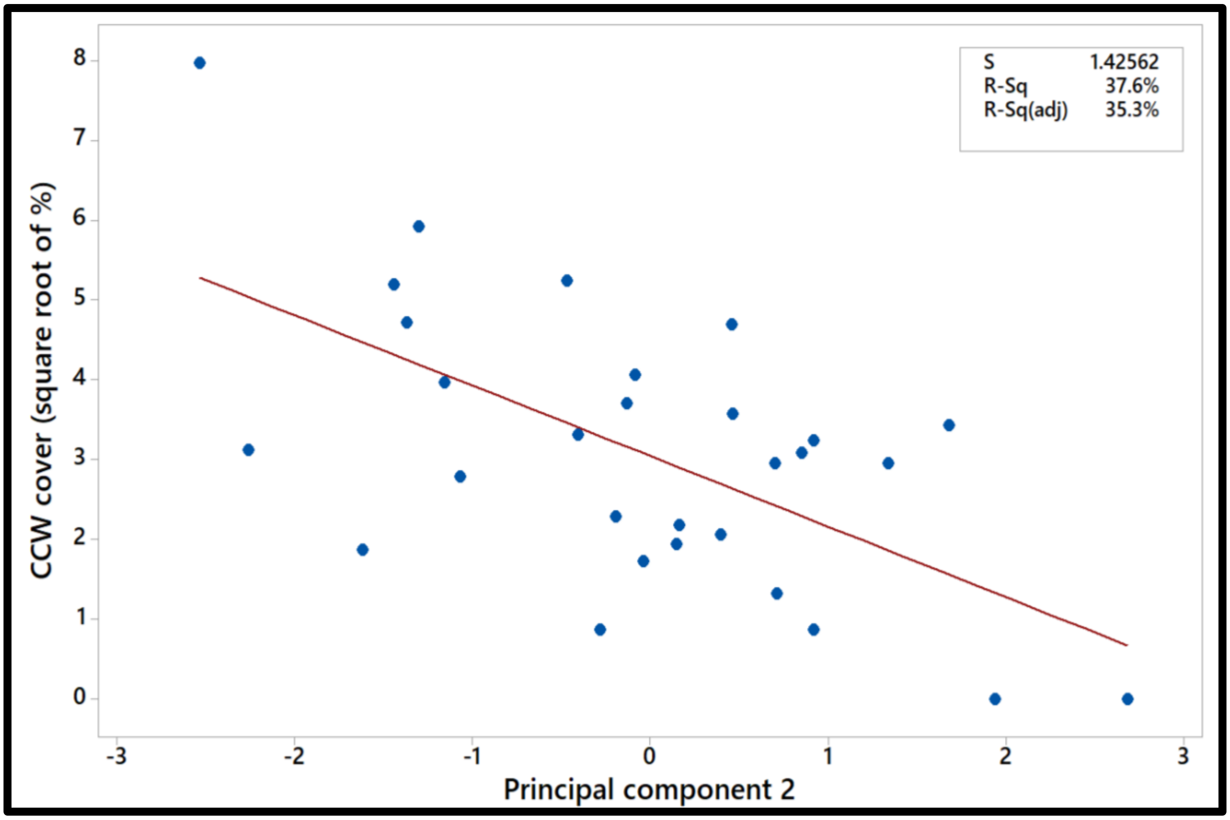

Figure 11. Linear regression of the square root of CCW cover (%) against the second principal component value from the PCA corresponding to each grid square with fitted trendline

3.3 *CCW distribution within grid squares*

In eighteen out of the twenty-eight surveyed grid squares where calculation of a Chi-squared statistic to test for randomness of CCW distribution was possible, there was a significant difference ($P < 0.05$) from a random distribution pattern (Table 12). Therefore, the results suggest that in the vast majority of cases, CCW was not distributed evenly on the scale of the 10m x 10m grid squares that were surveyed but instead occurred in patches occupying only parts of grid squares. Since none of the grid squares surveyed were adjacent to each other, it was not possible to assess the patchiness of CCW distribution at larger spatial scales.

Table 12. Results of test showing significant difference (*, **and***) for random distribution of CCW using a Chi-Squared Goodness-of-Fit (Produced using MiniTab v 17)

Grid square	Degrees of freedom	X^2 - value	P - value
1	2	45.6	***
2	2	106.4	***
3	2	374.2	***
6	2	3.4	0.178
8	2	30.2	***
9	2	13	***
10	2	73.9	***
11	2	2.2	0.338
12	2	82.3	***
13	1	4.9	**
14	1	0.1	0.731
15	1	6.6	**
16	2	31.0	***
17	2	7.0	**
18	2	4.1	0.129
19	2	12.8	***
20	2	52.8	***
21	2	40.8	***
22	2	72.3	***
23	2	4.9	0.085
24	1	5.9	*
25	2	1.1	0.588
26	2	2.3	0.318
27	2	78.5	***
28	2	7.5	*
29	1	0.5	0.488
30	N/A		
31	N/A		
32	1	0.2	0.631
33	1	0.2	0.631

3.4 *CCW compared by distribution category from the 2013 survey and habitat type*

There was a significant difference in the median % CCW cover between grid squares based on the RSPB distribution category that was assigned to them in the 2013 survey (Kruskal-Wallis test; H = 15.59, DF = 2, P<0.001) (Figure 12). Grid squares that were classified as having the most CCW in the 2013 survey ("common") also had a greater % CCW cover in the present study than those that were categorized as ("frequent"). The lowest % CCW cover was recorded in grid squares where CCW was reported as "sparse or absent" in 2013. There was no significant difference in median % CCW cover when grid squares were grouped by that habitat type in which grid squares were located, however (glade, path or ride) (Kruskal-Wallis test; H=1.44, DF = 2, P = 0.486) (Figure 13).

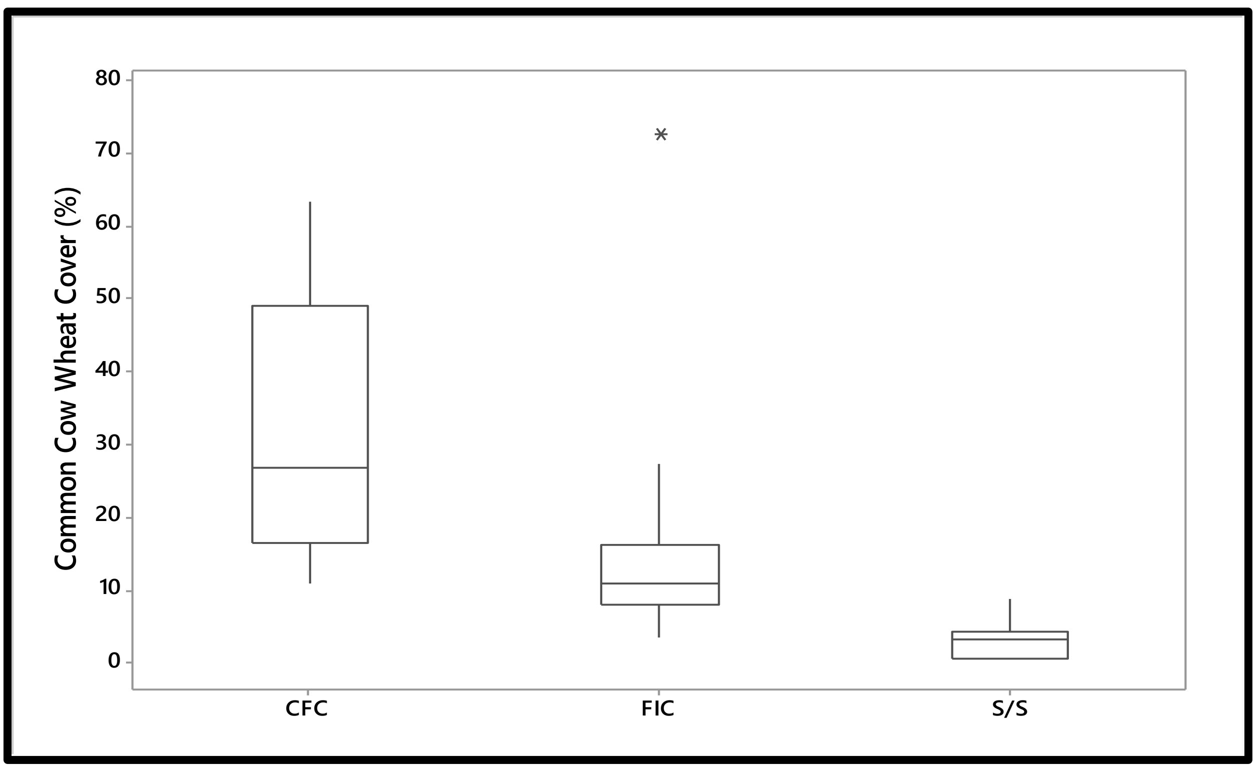

Figure 12. Comparison of CCW % cover and grid squares grouped by historical CCW abundance data based on 2013 RSPB distribution classifications (Produced using MiniTab v 17)

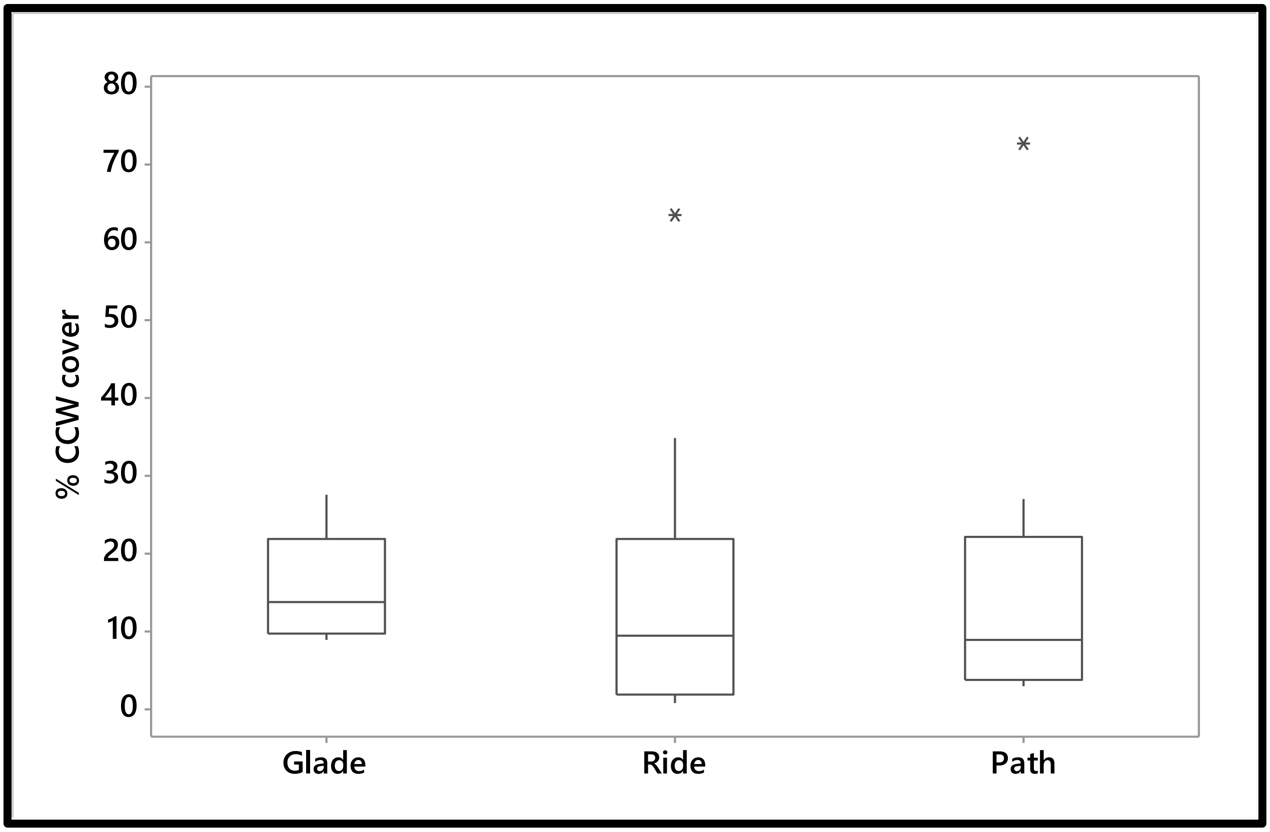

Figure 13. Comparison of CCW % cover and grid squares grouped by habitat (Produced using MiniTab v 17)

4. Discussion

The aim of this study was to investigate what variables if any, affect CCW distribution and abundance and if there are patterns in its distribution.

Oak sapling cover in a sampled grid square was the only variable among those recorded that had a significant effect on CCW abundance in sampled grid squares according to the multiple regression and stepwise regression models. In the backwards stepwise regression model, the % cover of oak saplings had a significantly positive effect on the % cover of CCW and in the multiple regression model, only % cover of oak saplings significantly predicted % CCW cover. This positive relationship also seemed to be reflected in the principle component regression, where principle component 1 captured oak associated effects, including the % cover of grid squares with oak saplings, and had a significant effect on % cover with CCW. This could be due to CCW utilising oak saplings in a hemiparasitic manner, with oak being one of CCW's supposedly favoured host species (Salonen *et al*., 2000).

However, this relationship could be due to both species preferring the same environmental conditions. For example, areas that have a medium to high % of canopy cover or semi-shaded areas may be preferable (as indicated by the collinearity of oak sapling cover and canopy cover reflected in the PCA loading plot). The relationship could simply reflect the underlying variable of how much vegetation was present in a grid square. The PCA loading plot suggests that bare ground cover and oak sampling cover may be negatively colinear (though this was not significant when directly correlating the two variables). Variables were negatively related to bare ground cover and canopy cover, but the regression model did not find these associations to be significant for CCW.

Humpage (2004) and Underdown (1995) found bare ground had a negative effect on the local abundance of CCW, but the present results do not support this finding. When Underdown (1995) investigated the effects of ground cover on CCW by testing the hypothesis that CCW would be more abundant in areas with moderate plant cover, this was supported by the data. However, for the secondary hypothesis (which species was the preferred host of CCW), results were inconclusive and there was no mention of oak saplings. There were, however, significant negative relationships found between CCW and two dominant species recorded in that study - bramble *Rubus fruticosus* and great woodrush *Luzula sylvatica*.

Humpage (2004) analysed the co-occurrence of CCW with different plants including trees, shrubs and vascular species, including oak, distinguishing between oak in the canopy (*Quercus* C) and oak on the ground (*Quercus* G) for the purpose of the study. Humpage suggested from the analysis that *Quercus* G (oak saplings) may exist in a similar ecological niche as CCW, thus having the same environmental preferences. Similar to how the PCA showed a positive relationship with oak saplings in the present study, *Quercus* G was more closely associated with CCW in the Humpage study.

Any hemiparasitism that is occurring where CCW exists could possibly be influencing the plant diversity in a grid square. McKibben and Henning (2018) suggest that hemiparasitic plants can regulate plant diversity by suppressing dominant species through the reduction of available nutrients in a local environment. Therefore, it could be possible that here CCW is suppressing dominant species within a grid square, thus benefiting CCW and creating greater plant diversity. Consideration must therefore be given to such hemiparasitic plants to be classed as ecosystem engineers and/or biodiversity indicators resulting in them being a conservation priority (Fibich *et al.*, 2017).

The overall species composition of a grid square is also influenced by other conditions such as soil pH, canopy cover, nutrient availability, and soil moisture (McKibben and Henning, 2018). Tesitel *et al*. (2015) examined hemiparasitic interactions when affected by nutrient availability and soil moisture. In times of nutrient and water stress the hemiparasite would extract nutrients and water from its host, to the detriment of the host. This serves as an example that abiotic variables can have an influence on plant species composition locally ($<100m^2$). It is interesting to compare the findings from this study in the Blean Woods NNR, which did not find any significant abiotic variable influence on CCW abundance, with that of Humpage (2004) who reports significant effects of soil pH and Ca concentrations on CCW presence and frequency. One possible reason could be that the surveyed areas in the two studies differed in location and/or habitat. Humpage does not specify which areas or habitats within the Blean Woods NNR were sampled. It is therefore possible that the survey areas used in this study were more diverse (glades, rides and paths), which may contain other unknown variables.

As already suggested, the significant relationship found between CCW and oak saplings could be an indication that CCW is dependent on this host in the Blean Woods NNR. Research on CCW in the Czech Republic growing under varying light levels during spring and summer revealed that CCW still showed a high rate of photosynthesis in comparison to non-parasitic plants species during the summer months due to its life cycle (spring emergence and rapid growth), but its carbon gains were very low (Svetlikova *et al*., 2018). This was shown to be a result of CCW being able to gain carbon through its hemiparasitic association with the host plant when canopy cover is greatest and illustrates the importance of the hemiparasitic relationship for CCW.

The statistical analysis revealed that CCW seemed to be distributed non-randomly and in a patchy pattern across most of the grid squares. This fits in well with existing literature, which describes CCW as dropping seeds close to the parent plant relying mainly on barochory for seed dispersal (Winkler & Heinken, 2007). The plant's lack of seed dispersal mechanisms with which to spread its progeny more widely may be why CCW had a clumped distribution on a very local scale (<100m^2) in the grid squares sampled for this study. If CCW had been more randomly distributed at a local scale, this could have indicated a greater effect of dispersal via myrmecochory (Masselink, 1980). Wood ant nests had a mean distance of 11.1m from the grid squares surveyed for this study, with the furthest recorded nest at 28.6m, which is well within the known maximum foraging range of 50m for wood ants (Wright *et al*., 2000). Although a clumped distribution of CCW was found in this study, wood ants from a nearby nest could disperse CCW seeds into previously un-colonised areas of woodland. Over time and given the right environmental conditions a pioneer plant could successfully produce seeds that would produce a new cluster of CCW, possibly supporting a future metapopulation of heath fritillary by an increase in CCW distribution. However, Winkler & Heinken's (2007) simulation of CCW dispersal using 15m x 15m – 225m^2 grid squares found that dispersal by myrmecochory only increased dispersal distances by 1-2 metres, which is not a sufficient distance to rapidly colonise areas opened up by management in a large woodland such as the Blean Woods NNR (500ha). Likewise, dispersal by barochory resulted in a mean distance of dispersal between 15-25cm from the parent plant. Their model suggested that CCW grows and spreads gradually from an original population with a limited distribution taking three years to disperse by only eight metres even when aided by myrmecochory, which would explain the locally clumped distribution at a scale of <100m^2 found in the Blean Woods NNR. This limited ability of CCW to disperse even short distances from one generation to the next is likely to create significant challenges for management that aims to increase its distribution in the Blean Woods NNR and elsewhere (Trakhtenbrot *et al*., 2005).

Management methods that can be used to increase dispersal of low-mobility species include facilitated dispersal and transplantation. Walter (2005) attempted to transplant CCW into previously uncolonized areas of the Blean Woods NNR, but without success. CCW turves where transplanted and seeds were sown to areas assumed suitable for CCW from existing areas in the Blean Woods NNR, however only a few seedlings matured. Despite plant and seed specimens originating from within the woodland, local conditions where they were planted/sown may not have been suitable. It is possible that transplanting CCW disrupts interactions such as hemiparasitism and/or mycorrhizal fungal associations existing at the source location that are not re-established at the transplant location. In contrast, Winkler & Heinken (2007) achieved better results from their artificial introduction in NE Germany possibly because they did not transplant but used only seeds where local above and below ground environmental conditions were more ecologically suitable for CCW.

As CCW is an autonomously germinating species (Tesitel *et al*., 2015), if a host cannot be found, seedlings may not survive or grow with deficiencies resulting in CCW not attaining its quality potential, which in turn can affect dependent species such as the heath fritillary. For example, Ca is important in cell wall formation and maintaining structural stability and is also used to maintain membrane structure and permeability (Campbell and Reece, 2008). Plants deficient in Ca can show signs of leaf deformity and reduced root growth (Soetan *et al*., 2010). Therefore, poor LHP quality could affect fecundity and growth rates in heath fritillary populations (Awmack and Leather, 2002), thus highlighting the importance of analysing LHP distribution and abundance and its immediate environment and whether management can maintain and/or increase it.

The comparison of CCW cover in grid squares based on abundance records from 2013 to 2018 showed that there seems to have been no significant change of CCW cover in these grid squares during the intervening five-year period. Grid squares in areas with high abundance (CFC or 4-5) in 2013 continue to have comparatively high CCW cover currently, possibly suggesting that either management is working well in these areas and/or historically CCW remains abundant only in certain areas of the wood due to the persistence of suitable ecological conditions. Having the required amount of CCW in the Blean Woods NNR is highly important for the continued successful conservation of the heath fritillary, as was found with another similar species. Smee *et al*. (2011) found significant increases in marsh fritillary larval webs where the LHP, *Succisa pratensis* was more abundant. Wheatley (2018) suggests that areas where CCW is at least frequent using Warren's abundance score (1985) are required to support the heath fritillary with the report revealing the butterfly to be existing in almost all areas in the wider Blean complex where cow-wheat cover is common.

Other species with similar LHP requirements as the heath fritillary include the glanville fritillary which selects suitable new habitats in which to oviposit close to where they emerged, provided it contains sufficient quantities of its LHPs (Hanski and Singer, 2001). The influences of gene flow in a local population of glanville fritillary is therefore driven largely by the female's choice of 'suitable' habitat where a preferred LHP is chosen over the other. Warren (1987a) found similar behaviour with ovipositing heath fritillary females, resulting in heath fritillary larvae in woodland habitats utilising CCW due to its greater abundance over *Plantago lanceolata* and *Veronica chamaedrys*. However, in areas where CCW is sparse, this could delay larval growth, due to the larvae having to roam further between LHPs. Thus, having frequent to abundant quantities of CCW ultimately benefits the survival of larvae and could result in an earlier emergence. This means that – despite its limited ability to spread on its own accord - local spread of CCW must be encouraged in addition to creating suitable habitats for CCW to colonise.

Although not a significant relationship, an observation from the results in this study was that CCW was surveyed in grid squares mostly with a soil pH of between 4-5, with most of the samples centered around a pH of 4.4, matching the findings of Humpage (2004), who, reported a significant relationship between pH and CCW presence. Humpage (2004) reports that CCW was present when pH was between 4.3 and 4.5 and absent when pH was below 4.3. This study found that CCW was present at low soil pH levels with nine grid squares recorded with a soil pH of <4.3 all containing CCW. CCW was also present at higher soil pH levels of >5. It is therefore possible that soil pH is not as limiting a factor to CCW presence as previously thought and that most of the areas surveyed in the Blean would, in principle, have soil pH conditions suitable for CCW. This information could be useful in identifying areas where CCW could be encouraged through management. For example, coppicing could focus on areas with a suitable soil pH (4.4) close to existing CCW where it could gradually spread.

Frouz and Jilkova (2008) mention that ants have an ability to make acidic soils more neutral and neutral soils more acidic around the nest periphery, possibly by their movement of organic matter altering soil chemistry, but CCW distance to ant nest was not shown to significantly affect CCW cover in grid squares in this study. However, the PCA suggested that CCW abundance increased with greater distance to ants' nests.

Currently, the Blean complex acts as a refugium for the heath fritillary in the South-East of England. Ecological forecasts for natural refugia in the South-East of England, including the effects of climate change, mainly predict pressures on water availability from increased urbanisation and long dry summers and localised flooding from extreme precipitation events (Suggitt *et al*., 2014). In the event of a prolonged dry period, the current management in the Blean Woods NNR can reduce the pressure on water availability by blocking the drainage of existing wet areas (Nash and Johnson, 2014). However, forecasts specific to the Blean complex would include the increased possibility of extreme weather events that may cause wildfires due to periods of warm, dry weather and localised flooding after heavy rainstorms (Suggitt *et al*., 2014). Areas of the Blean refugium where this could occur are areas of dry heath and gorse and low-lying areas near to streams on the slow draining clay (Nash and Johnson, 2014). In addition, the impacts of an extreme climate event, such as the 1987 storm can have very local effects ($<100m^2$) on certain species both floral and faunal. For example, downed dead wood can change soil composition locally with certain species benefitting from the newly open canopy and nutrient enriched soil from the decaying wood (Smart *et al*., 2014). These effects also need to be considered when producing management prescriptions as leaving a decaying tree in its place may encourage greater biodiversity locally but alter the biotic and abiotic interactions such as encouraging avian species that feed on larvae and increasing/decreasing soil pH that could negatively affect pre-existing species such as the heath fritillary and CCW. Direct effects on the species could result from seasonal changes in both temperature and precipitation causing milder winters and wetter summers, which are predicted (Suggitt *et al*., 2014). Butterflies are sensitive both to climatic changes and alterations (both positive and negative) to their habitats with specialist species such as the heath fritillary most affected (Menendez *et al*., 2007). Wheatley (2018) also mentions climate as a factor in heath fritillary success year on year, where wet summers can cause a decline in numbers. LHPs can also be affected by climate change and

Kharouba and Vellend (2015) point out that the timing of emerging LHPs and flowering nectar food sources can be important for butterfly species with both larval and adult stages of the life cycle. This timing could be intrinsically altered by climate change possibly requiring the co-dependent species to adapt to a changing climatic environment.

The apparent lack of deer in the Blean woodland complex currently dictates the present management practice of coppicing as there are no natural checks on the fast-growing vegetation (Bourn *et al*., 2012). The data presented here indicate CCW reoccurs in open areas that do not undergo significant succession. However, the succession from open areas to dense scrub is advantageous to other rare faunal species in the Blean. For example, the nightingale *Luscinia megarhynchos* breeds in dense scrub and coppice regeneration, which larger areas of woodland can accommodate, such as the Blean (Perrins and Overall, 2001). This highlights a potential conflict between management priorities such as CCW and the heath fritillary and the nightingale, each with conflicting habitat requirements (Nash and Johnson, 2014). This could be particularly challenging as CCW seems to require open, connected and contiguous habitats to spread into in the long term due to its poor dispersal.

At present no deer species have been recorded in the wider Blean complex (Bourn *et al*., 2012; pers. obs., 2018), but an expanding population to the south could soon cross a major highway and colonise the wood (Holmes and Wheaten, 2002). This potential colonisation could possibly lead to a change from the current woodland management practice of coppicing, as deer herbivory would reduce new growth and existing scrub, therefore preventing natural succession across the woodland (Davalos *et al*., 2014). Over time this natural form of woodland management could achieve the same results as coppicing, which may benefit CCW but be detrimental to other species such as the nightingale (Holt *et al*., 2011). However, the conservation managers can plan for this eventuality by setting aside areas for avian species with deer exclusion fencing, already on trial in the Blean Woods NNR (pers. obs., 2018). It is of course a possibility that deer may also graze on CCW and other herbaceous woodland plant species, which may require areas where they occur to be protected by fencing as well (Davalos *et al*., 2014). A more economical option, if only as a deterrent would be to use ‘dead hedges’ made from cut branches laid horizontally, protecting young growth in newly coppiced areas (Fuller and Peterken, 1995).

Although there was no significant difference in CCW cover between the three habitat types that were sampled for CCW (glades, rides and paths), they all contained significant amounts of CCW that are likely to persist and have the potential to gradually spread, as long as the management caters for these open spaces (Fuller and Peterken, 1995). Whilst it is understood that species such as the heath fritillary have natural year on year fluctuations due to biotic and abiotic variables, some of which are out of direct human control (man-made climate change being an indirect variable) there is still much conservation managers can do to reverse their declines and stabilise and increase their populations (Chandler *et al*., 2016). Although the heath fritillary is categorised as Least Concern by the IUCN in continental Europe (Fox *et al*., 2010), this UKBAP species (Holloway *et al*., 2003) requires anthropogenic interventions in the UK and it is recommended that the results from this study and from those previously carried out on both CCW and the heath fritillary will be used when decisions are made on the future conservation management of the two species. Prescriptive methods for the continued successful conservation of the heath fritillary must include managing for its LHP (CCW), which primarily includes the provision of suitable habitat. While coppicing has been seen to be successful in providing this habitat, concerns over coppicing frequency and the number of hectares coppiced were raised by Hodgson *et al*. (2009). Their simulation model predicted (in a worst-case scenario) that the then observed rates of coppicing in the wider Blean complex would need to be doubled to meet UK BAP targets for the heath fritillary. However, these targets seem to have been met with encouraging reports in recent years (Bourn *et al.*, 2012; Wheatley, 2018). This must be aired with caution as Fuller and Peterken (1995) stated that this method is labour intensive and can become costly, with no guarantee that it can be continued long-term, mainly because of the finite financial resources available to all conservation groups, including those active in the Blean (Begon *et al.*, 2006).

Ojanen *et al*. (2013), called for the continuation of long-term butterfly and LHP studies in a large area (such as the Blean complex), with smaller local studies within (an area of Blean Woods NNR). This is already the case in the wider Blean complex, with various conservation groups running organised surveys and recording heath fritillary populations in their respective woodlands (many of which are volunteer led) which is overseen, compiled and reported by Butterfly Conservation (Wheatley, 2018). However, there is possibly more that can be done by using citizen science to survey LHPs incorporated into existing butterfly transect walks using the abundance score method by Warren (1985). Citizen science has grown in the last decade, partly due to conservation groups recruiting and training volunteers to help with collecting vast amounts of species distribution and population data to inform management plans and to assess the state of a species. (Chandler *et al*., 2016).

Humpage (2004) urged caution when reading past literature on CCW ecology in relation to heath fritillary conservation, as locally distributed CCW populations can have different ecological requirements in contrasting habitats, such as woods, scrub, heaths and moorlands up to 960 metres (pers. obs., 2018). In Exmoor, CCW can be found on open heathland and recently coppiced woodland in sheltered combes where it parasitises bilberry *Vaccinium myrtillus*. In Kent and Essex, bilberry does not occur, but other local environmental factors exist to provide suitable habitat, such as woodland ride networks and sunny glades. Therefore, investigations into the local ecology of CCW should be encouraged to find similarities between these geographically distinctive habitats, which in turn can inform management decisions across the different heath fritillary habitats. An example of this phenomena occurs in another similar species. Pschera and Warren (2018) studied the habitat selection of the marsh fritillary and found the species' needs varied geographically in a similar way to the heath fritillary in different habitats across southern England. It can be seen however, that one common factor in all the sites in southern England where both CCW and heath fritillary occur is the acidic conditions of the soil (Humpage, 2004).

From the findings in this and other localised studies/surveys on CCW (Hodgson *et al*., 2009; Humpage, 2004; Walter, 2005, Wheatley, S. (2018, 2019, 2020), site-specific prescriptive measures are recommended for the management of CCW in the Blean Woodland complex. Firstly, although labour and cost intensive, rotational coppicing is a key management practice that creates new areas of colonisation for CCW, where it exists with nearby with oaks. Secondly, maintaining existing woodland ride networks and sunny glades with CCW and oaks present would aid in its gradual spread. Thirdly, but no less important, regular surveys (every 2-3 years) of CCW distribution must be carried out to assess the habitat management carried out in the autumn/winter months and the success of the spring/summer flowering seasons. Due to its gradual spread, surveying CCW in areas where it has previously existed would be beneficial with particular focus on whether CCW has expanded its ranged or remained stable. In addition, conservation managers could organise oak sapling surveys along rides and path edges where CCW exists to add further insight into the possibility that oak saplings are parasitised on by CCW. Without these distribution surveys conservation managers would not be able to plan future management or evaluate its results. Therefore, encouraging citizen science and recruiting/retaining volunteer surveyors is an important prescriptive measure considering the size of the Blean Woodland complex.

5. CONCLUSION

The data presented here suggests that CCW may be associated with oak saplings, possibly due to parasitising them. This relationship should therefore be taken into consideration in present and future management plans in the Blean woodlands, especially in areas where mature oak is present and can act as a source for oak seedlings in the understory. Also important are the findings that seem to show CCW distributed non-randomly and in a patchy pattern in many of the surveyed grid squares. This provides further evidence of CCW's poor dispersal at medium and long range that does not seem to be significantly aided by ants. Therefore, a long-term management plan facilitating and allowing for gradual CCW spread and movement distribution into suitable habitat is required. This spread would be best supported by ensuring that habitat suitable for CCW remains stable and well-connected throughout the Blean. In addition, the comparison of CCW cover in grid squares based on abundance records from 2013 and 2018 seemed to show no significant change of CCW cover in these grid squares. This information should aid the woodland managers as to which areas are most suitable to maintain and increase CCW distribution and abundance in areas where it is already seemingly stable.

It is recommended that further study is conducted on CCW and its distribution and spread within other woodland areas of the wider Blean complex in East Kent and in Essex with more localised studies into CCW distribution required in areas where the heath fritillary is present. This could help to provide localised solutions for CCW management and provide more robust support for the variables identified by Humpage (2004) and in the present study that may affect CCW distribution. Although not used in this study due to time constraints, it is hoped the development and use of a predictive distribution model, possibly using similar variables, would be also be advantageous for the management of the species. Creating a model would have involved collecting more data on the variables used including soil parameters throughout the Blean Woods NNR and would have been too time consuming to carry out in the time frame. Also, only a small proportion of the Blean Woods NNR is considered suitable habitat for CCW (rides, paths, and glades). This would have meant modelling would have been restricted to these areas of the Blean Woods NNR, and no GIS data currently existed at the time of writing to assist in this. It is hoped that the findings of this study will also be useful in the conservation efforts for the south-west populations of heath fritillary.

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