The influence of canopy cover on nest size and distribution of the red wood ant *Formica rufa* L. at its northern limit

David Lamin

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Abstract

- The current distribution of *F. rufa* populations at the species' northern limit was established through systematic surveys of three key sites. The results show populations are largely concentrated within Gait Barrows and Arnside Knott, whilst nearly absent from Eaves Wood.
- The influence of canopy cover on nest distribution was found to be significant overall with increased canopy cover linked to nest decline and abandonment. However, within-site analysis revealed contrasting relationships between canopy openness and nest status across populations, which have been attributed to differences in site characteristics.
- An overall relationship between reduced canopy openness and active nest mound size was identified but within-site analysis revealed this relationship to be weak. The results are considered to support previous findings that the species shows a distinct preference for woodland edge habitat.
- Changes to the northern distribution were analysed via survey records and reports. This revealed a marked decline in active nests within all populations in the decade prior to the study. Gait Barrows and Arnside Knott populations have since shown signs of a partial recovery but still appear to be weakened and contracted; whilst the Eaves Wood population appears close to extinction.

1. Introduction

Red wood ants, widely referred to simply as 'wood ants', belong to the subgenus *Formica sensu stricto* (*F. rufa* group) containing several species of morphological and ecological similarity whose distribution extends throughout the Holarctic region (Stockan et al., 2016). Regarded as keystone species, wood ants influence woodland ecosystems in a variety of ways: playing an important role in seed dispersal (Gorb & Gorb, 1999); serving as biological control agents in the suppression of pest species (Olofsson, 1992); and significantly affecting nutrient cycling in the woodland environments that they inhabit via the transportation and deposition of organic material in the process of building nest structures (Frouz et al., 2016).

Nest structures are typically dome-shaped mounds consisting of accumulations of organic material such as twigs, leaves, needles and grass stems, sometimes referred to as 'thatch' (Risch et al., 2016; Robinson & Robinson, 2008). In addition to acting as a hub for the wood ant colonies themselves, nest mounds support a range of other organisms, known collectively as myrmecophiles, through the provision habitat and food (Robinson & Robinson, 2013).

The effects of climate change are considered to threaten wood ant populations as phenological changes alter food availability at certain times of year and elevated over-winter temperatures lower survival rates and productivity in workers through increased activity during typical periods of dormancy (Sorvari et al., 2011). As climate change continues populations at the edge of distribution ranges are thought to provide insight into the ability of species to maintain or expand their current range (Rehm et al., 2015), making the study of these populations particularly important.

Since the mid 20th century, the northern limit of *Formica rufa* L. in Britain has shifted south from northern Cumbria in North West England (Collingwood & Satchell, 1956) to within the Arnside and Silverdale Area of Outstanding Natural Beauty (AONB) on the northeast corner of Morecombe Bay (Robinson, 2001); a change which seemingly goes against the general trend of a northward shift in distribution of species in the northern hemisphere (Häder & Barnes, 2019). The former existence of colonies north of Morecombe Bay has previously been attributed to the late 19-century practice of wood ant introductions as supplementary food for pheasant rearing activities, suggesting the now extinct Cumbrian *F. rufa* populations occurred outside of the species' natural climatic range thus providing an explanation for their eventual disappearance (Robinson, 2001).

Hughes (1975) identified a historic link between the British distribution of *F. rufa* and coppicing, a formerly widespread woodland management practice that declined sharply throughout the 20th century (Kirby et al., 2017). The resumption of coppicing on several sites in the Arnside & Silverdale AONB in the late 20th century, primarily for the benefit of butterfly species such as the Duke of Burgundy *Hamearis Lucina* and High Brown Fritillary *Argynnis adippe,* has been credited with maintaining the stability of some northern *F. rufa* populations into the early 21st century (Robinson, 2001; Robinson & Robinson, 2008). Over subsequent years, however, declines have been reported through independent and largely asynchronous site monitoring in the region (C. McCoy, G. Swainson & E. Robinson pers. comm., 2019), thus requiring further investigation.

Wood ants require a stable microclimate within their nest mounds that is consistently warmer than ambient temperature during the active period (typically March to September), in order for broods of workers and sexual offspring to develop, achieved through thermoregulation (Frouz et al., 2016). Risch et al. (2016) propose that nest mound thermoregulation involves a combination of the following processes: insolation warming the nest surface directly; heat transfer via workers basking in direct sunlight and entering the nest; metabolically generated heat via activity within the nest; and heat produced as a consequence of the decomposition of organic matter from which the nest is constructed.

Nest mound size has been found to positively correlate with worker numbers in a colony (Sorvari & Hakkarainen, 2005), with larger nests thought to be less reliant on insolation for thermoregulation and therefore more shade tolerant (Risch et al., 2016). Increasing canopy cover has been found to result in localised thermal environments that are both cooler and more stable than those of more open areas, with positive relationships found between nest mound size and increasing canopy cover for some species such as *F. lugubris* in Britain (Chen & Robinson, 2014) and *F. aquilonia* in Finland (Sorvari & Hakkarainen, 2005; Kilpeläinen et al., 2008). Consequently, it could be expected that active *F. rufa* nests located in areas of increased canopy cover will be larger than those in more open areas. However, excessive shading is also considered to be one of the major drivers of nest abandonment and relocation in *F. rufa* (Robinson, 2001) suggesting there may be an optimum range of canopy openness for nest sites.

The ability of wood ant colonies to successfully relocate and multiply is strongly affected by colony structure (Risch et al., 2016). Polygynous colonies (those with more than one queen) rely primarily on colony budding involving reproductive females, known as gynes, establishing a separate nest with workers from the natal nest; these nests can sometimes remain socially connected in a state known as polydomy (as opposed to monodomy where colonies comprise a single nest) (Maeder et al., 2016). In monogynous colonies (those with only one queen) new gynes are predisposed to undertake dispersal flights once inseminated and utilise temporary social parasitism as a reproductive strategy, a process which involves a gyne entering the nest of another species, usually of the subgenus *Serviformica,* to depose its queen before enlisting the host colony workers to rear the first brood (Maeder et al., 2016).

Robinson & Robinson (2008) suggest that colony budding constitutes the main reproductive strategy of *F. rufa* at its northern limit in Britain, with newly established nests often occurring within the same territory as the natal nest resulting in short-range dispersal and inter-nest competition. The reliance on this strategy is considered to mean that these populations are less likely to successfully relocate and colonise new areas if conditions in their current location become unfavourable due to the lack of long-range dispersal abilities (Robinson, 2001). Under stable conditions, active nests have been known to persist in the same location for more than 20-years; in such cases the nest site often possesses a combination of high insolation and some degree of canopy cover providing shelter from precipitation (Robinson & Robinson, 2008). Nests have also been found to regularly make use of additional elevation offered by rocks and tree stumps (Robinson & Robinson, 2008) and most commonly have an aspect between south and west (Risch et al., 2016).

This study seeks to evaluate the current status of *F. rufa* at its northern limit in Britain whilst investigating potential relationships between canopy cover and distribution characteristics through addressing the following:

- What is the current distribution of *F. rufa* within three key sites at the species' northern limit?
- Does canopy cover influence nest distribution?
- Is there a relationship between canopy cover and active nest mound size?
- How has the northern distribution changed over time?

2. Methods

Table 1. Study sites selected based on historic records of the largest recorded *F. rufa* populations at the species' northern limit in Britain. *Including Heathwaite (23.8 ha of survey area).

Site	Location	Elevation (m)	Survey Area (ha)	Last survey
Arnside Knott	SD455773	110	67.2*	2016 (National Trust)
Eaves Wood	SD465762	60	52.3	2008 (National Trust)
Gait Barrows	SD482773	35	47.6	2016 (E. Robinson)

2.1. Study Sites & Timing

To study the effects of canopy cover on *F. rufa* colonies and examine changes to populations at the species' northern limit, three sites with historic records were surveyed within the Arnside and Silverdale AONB (Table 1). All three sites feature mixed limestone woodland components that have been consistently managed through coppicing on rotation throughout the periods between surveys (C. McCoy & G. Swainson, pers. comm., 2019). Despite their close geographical proximity (Fig. 1.), the three sites differ in topography and quantity of woodland cover.

Surveys were carried out between June-July (2019) to enable confirmation of active nests and to ensure canopy cover was stable (Chen & Robinson, 2014). In addition to previously surveyed areas, the 2019 Arnside Knott survey included a section of adjacent land known as Heathwaite, at the request of the National Trust who own and manage the site.

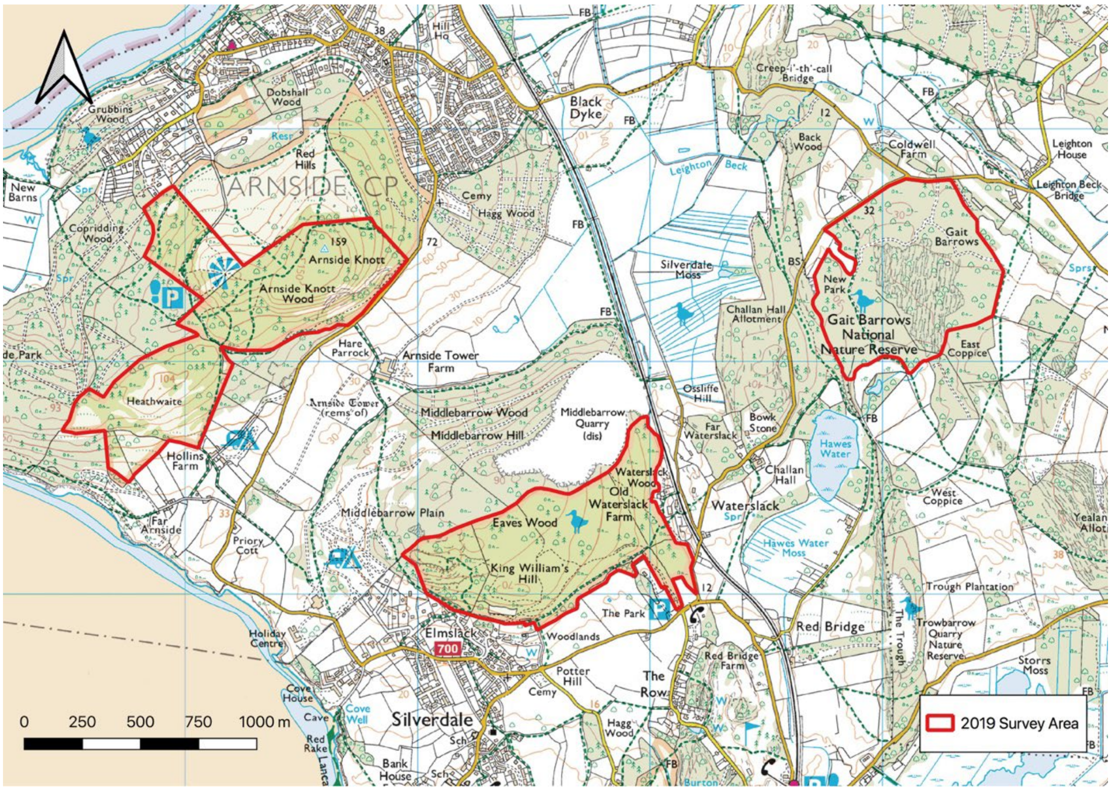

Fig. 1. Study Sites – 2019 survey areas (outlined in red) for Arnside Knott with Heathwaite (left), Eaves Wood (middle) and Gait Barrows (right)

2.2. *Nest Location*

Nests were located by systematically covering all accessible areas within each site on foot whilst scanning the ground for signs of activity and nest structures (methods recommended by E. Robinson, pers comm., 2019). The grid reference and elevation data for each nest site was determined using a Garmin eTrex 20 handheld GPS receiver (minimum accuracy of 10m). Nest aspect was determined based on the local topography and adjacent features of each nest site and measured using a Suunto A-30 compass.

2.3. *Nest Status*

Each located nest was given a unique ID and categorised according to its status and structure. Active nests were defined by the presence of workers on the nest surface (Robinson & Robinson, 2008) and put into one of two categories: active without mound (activity centred around a patch of ground or tree stump without a mound structure) or active mound (distinct active mound structure) with a third category for inactive nests (mound structure with no *F. rufa* workers present).

Instances of inter-nest activity, foraging trails and presence of nest associate *Formicoxenus nitidulus* (BAP Priority Species) were also recorded when observed.

2.4. Nest Measurements

Nest mound height and two basal measurements (longest diameter and perpendicular diameter) were taken for each active nest mound (Chen & Robinson, 2013). Above-ground nest volume was calculated using the equation for a hemi-ellipsoid: $V = (\frac{4}{3}\pi abc)/2$ (Sorvari et al, 2016). Basal measurements of inactive nests were also recorded to assist with analysis of historic survey data.

2.5. Canopy Photography

A full-frame DSLR camera (Canon 5D MKII) fitted with an 8mm lens (Canon EF 8-15mm f/4L Fisheye USM) was used to capture circular 180º images above each nest. For each image the camera was tripod-mounted and positioned 25cm above the highest point of the nest pointing skyward. The top of the camera was oriented north using a compass and levelled to horizontal. A remote shutter release was used to ensure persons did not appear in the image. Consistent camera settings were used throughout (focal length: 8mm; aperture: f/8.0; ISO: 100; focusing distance: ∞) with aperture priority mode used to achieve consistent exposure levels.

2.6. Canopy Analysis

Canopy images were analysed using Gap Light Analyser (GLA) Version 2.0 software (Frazer et al., 1999) to calculate the percentage of canopy openness for each nest site. As the images were oriented using a magnetic compass at the point of capture, magnetic declination was calculated (North = 0˚ 45min west) based on a central point between the three sites for the period of June and July 2019 (British Geological Survey, 2019) and inputted into the software settings prior to canopy analysis. Threshold adjustments and masking were necessary for some images to ensure reflective surfaces (e.g. drystone walls and tree bark) were not interpreted as open sky by the software.

2.7. Mapping and Nest Density

GIS mapping was carried out for each site using data from the June-July 2019 surveys and historic survey data in QGIS Version 3.8.2-Zanzibar (QGIS Development Team, 2019). Nest density, defined as the number of active nests within 100m of every active nest (Sorvari et al, 2016), was calculated for all surveys in QGIS using the geoprocessing tool 'Buffer' to create 50m buffers around each active nest. The 'Join Attributes by Location' tool with 'overlap' selected was then used to calculate the number of overlaps for each buffer and thus the number of active nests within 100m of each focal nest.

2.8. Data Analysis

Prior to testing, two nests were excluded from canopy analysis; the first due to dense vegetation above the nest surface preventing a representative image being captured (Arnside Knott); the second due to its atypical location within a covered storage shed (Gait Barrows).

Statistical analyses were carried out using the 'stats' package (R Core Team, 2018) in RStudio statistical software (Version 1.1.456) (RStudio Team, 2016).

Canopy openness data was tested for normality (Shapiro-Wilk test) and homogeneity (Bartlett test), followed by one-way ANOVA to test variation in canopy openness between the three nest status categories. An overall test was carried out on canopy openness variation between the three nest type groups using data from all three sites: active without mound (n=36), active mound (n=99), and inactive (n=28). Separate tests for within-site variation were also carried out for Arnside Knott: active without mound (n=19), active mound (n=42) and inactive (n=10), and Gait Barrows: active without mound (n=16), active mound (n=56) and inactive (n=13). Surveys yielded an insufficient number of samples for within-site analysis of Eaves Wood.

Nest volume data was tested for normality (Shapiro-Wilk test) and homogeneity (Bartlett test) and consequently transformed by $\log_{10}$ to normalise distributions. To analyse variation in nest size at differing levels of canopy openness, nests in the active mound category were divided into three groups according to percentage of canopy openness (Chen & Robinson, 2014). Consistent boundaries were set to balance the groups as much as possible for three separate tests: one overall test including nests from all three sites and two within-site tests using nest data from Arnside Knott and Gait Barrows respectively (insufficient data for within-site analysis of Eaves Wood). Canopy openness groups for the three tests were as follows - All Sites: below 36% (n=35), 36–50% (n=30) and above 50% (n=34); Arnside Knott: below 36% (n=12), 36–50% (n=15) and above 50% (n=15); Gait Barrows: below 36% (n=22), 36–50% (n=15) and above 50% (n=19). One-way ANOVA was used to compare variation in nest volume between the three groups in each analysis.

3. Results

3.1. Current status of F. rufa within three key sites at the species' northern limit in Britain

A total of 165 nest were located during surveys of the three sites (Table 2), 137 of which were active. Gait Barrows had the highest number of active nests (73), the highest mean active nest density (19.9; SD: 9.0) and the highest mean active nest volume (0.054 m^3; SD: 0.107). Arnside Knott with Heathwaite had the second highest number of active nest (62), the second highest mean nest density (10.6; SD: 6.3) but the lowest mean nest volume (0.038 m^3; SD: 0.073). Eaves Wood had the second highest mean nest volume (0.043 m^3; SD: 0.043) but the lowest number of active nests (2) and the lowest mean nest density (1.0; SD: 0.0).

Table 2. Survey results June-July 2019. 'Mean Density' refers to all active nests (no mound and active mound); 'Mean Volume' refers to active mound nests only. *Nest data for Arnside Knott includes those found within adjacent are known as Heathwaite.

Site	Nests Found	Active Nests	Inactive Nests	Mean Density		Mean Volume	
	N	N	N	Within 100m	SD	m^3	SD
*Arnside Knott**	72	62	10	10.6	6.3	0.038	0.073
Eaves Wood	7	2	5	1.0	0.0	0.043	0.043
Gait Barrows	86	73	13	19.9	9.0	0.054	0.107
Overall	165	137	28	15.4	9.2	0.047	0.091

3.2. Canopy openness

Of the three sites (Table 3), Arnside Knott with Heathwaite demonstrated the highest maximum (74.74%), the lowest minimum (4.91%) and the highest mean canopy openness (45.32%; N=71; SD: 17.33). Gait Barrows had the second highest maximum (72.45%), the second lowest minimum (7.91%) and the second highest mean canopy openness (42.56%; N=85; SD: 14.73). Eaves Wood had the lowest maximum (50.75%), the highest minimum (8.14%) and the lowest mean canopy openness (22.95%; N=7; SD: 13.56).

3.3. Canopy cover and nest distribution

Canopy openness differed significantly between nest status groups across all three sites (One-way ANOVA: $F_{2,160}$=7.4, P=<0.001). Post-hoc testing (Tukey HSD) revealed canopy openness was significantly lower in the 'inactive mound' group compared to the 'active without mound' (P=<0.001) and 'active mound' (P=<0.05) groups (Fig. 2).

Table 3. Canopy openness results for all nest types found ('active without mound', 'active mound' and 'inactive') at the three survey sites in June-July 2019. Two nests were excluded from the analysis due to unrepresentative images. *Nest data for Arnside Knott includes nests found in Heathwaite, an adjacent area not previously surveyed.

Site	Nests	Max	Min	Mean

	N	%	%	%	SD
*Arnside Knott**	71	75.74	4.91	45.32	17.33
Eaves Wood	7	50.75	8.14	22.95	13.56
Gait Barrows	85	72.45	7.91	42.56	14.73
Overall	163	75.74	4.91	42.92	16.40

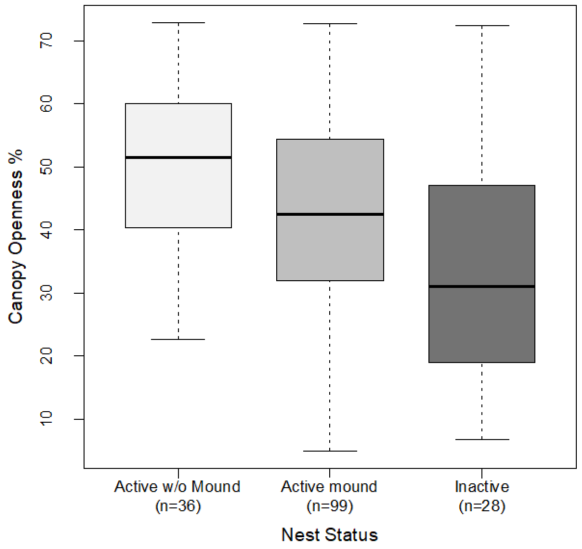

Fig. 2. The relationship between canopy openness and nest status across all sites: Differences between nest status groups across all three sites were significant (One-way ANOVA: $F_{2,160}$=7.4; P=<0.001) with canopy openness significantly lower in the 'inactive mound' group compared to the 'active without mound' (Tukey HSD: P=<0.001) and 'active mound' (Tukey HSD: P=<0.05) groups.

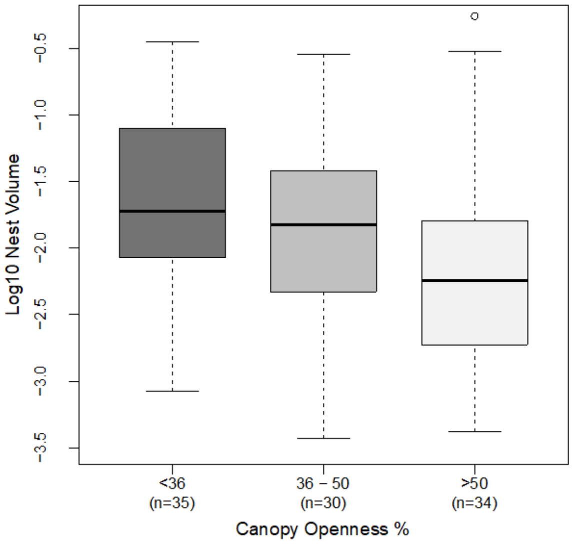

Fig. 3. The relationship between canopy openness and active nest mound volume across all sites: Differences between canopy openness groups consisting of nests from all three sites were significant (One-way ANOVA: $F_{2,96}$= 4.4; P=<0.05) with nests in the <36% canopy openness group significantly larger than those in the >50% group (Tukey HSD: P=<0.05).

Difference in canopy openness between nest status groups was also significant for Arnside Knott with Heathwaite nests (One-way ANOVA: $F_{2,68}$=9.2, P=<0.001). Post-hoc testing (Tukey HSD) revealed canopy openness differed significantly between all three groups with nests in the 'inactive' group displaying significantly lower canopy openness than both the 'active mound' (P=<0.05) and 'active without mound' (P=<0.001) groups (Fig. 4. A).

A significant difference was also shown between the two active nest groups with canopy openness significantly higher in the 'active without mound' group compared to the 'active mound' group (P=<0.05). No significant difference in canopy openness was shown between Gait Barrows nest groups (One-way ANOVA: $F_{2,82}$=0.1, P=0.90; Fig. 4. B).

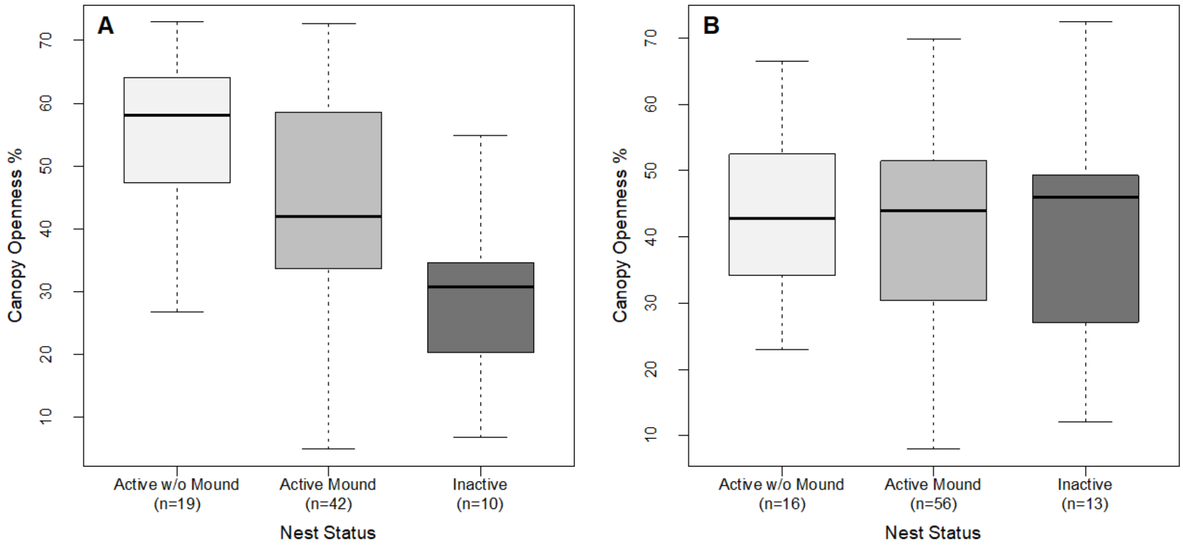

Fig. 4. The relationship between canopy openness and nest status: Differences between nest status groups for Arnside Knott with Heathwaite (**A**) were significant (One-way ANOVA: $F_{2,68}$=9.2; P=<0.001) with nests in the 'inactive' group displaying significantly lower canopy openness than both 'active mound' (Tukey HSD: P=<0.05) and 'active without mound' (Tukey HSD: P=<0.001) groups. Canopy openness was also significantly higher for nests in the 'active without mound' group compared to the 'active mound' group (Tukey HSD: P=<0.05). No significant difference was found between nest status groups for Gait Barrows (**B**) (One-way ANOVA: $F_{2,82}$=0.1; P=0.90).

3.4. Canopy cover and active nest mound size

Active nest mound volume differed significantly between canopy openness groups that included nests from all three sites (One-way ANOVA: $F_{2, 96}$=4.4; P=<0.05) with a high outlier present in the >50% canopy openness group (Fig. 3). Post-hoc testing (Tukey HSD) revealed nests in the <36% canopy openness group were significantly larger than those in the >50% group (P=<0.05). Nest volume did not differ significantly between canopy openness groups in separate tests for Arnside Knott with Heathwaite (One-way ANOVA: $F_{2,39}$= 2.6; P= 0.08) (Fig. 5. A) and Gait Barrows (One-way ANOVA: $F_{2,53}$= 2.4; P=0.10) (Fig. 5. B) with a high outlier present in the >50% canopy openness group of both sites.

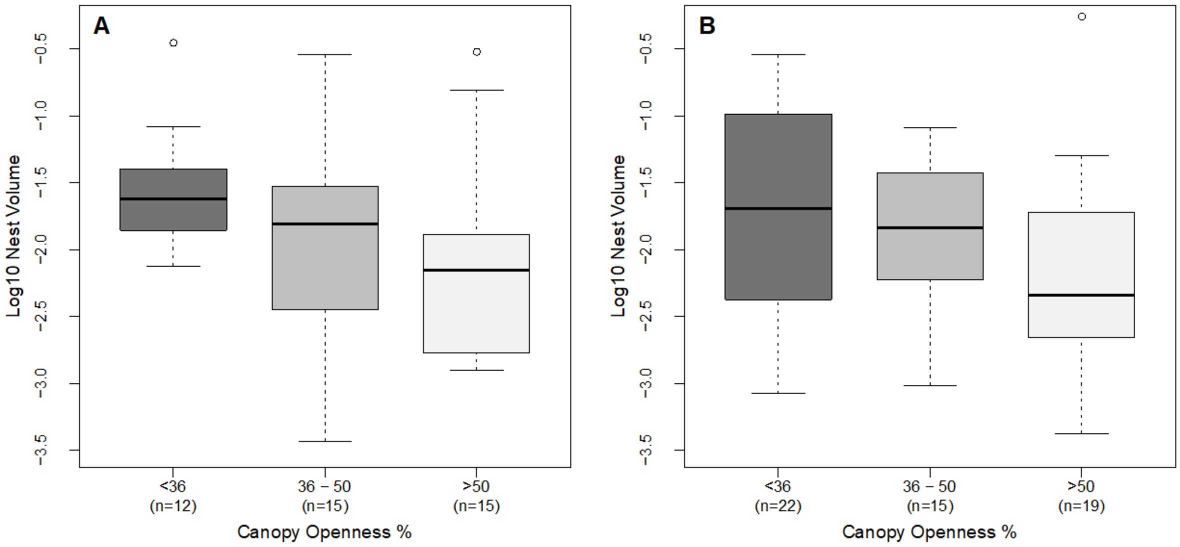

Fig. 5. The relationship between canopy openness and active nest mound volume: No significant difference was found between canopy openness groups for either Arnside Knott with Heathwaite (**A**) (One-way ANOVA: $F_{2,39}$= 2.6; P= 0.08) or Gait Barrows (**B**) (One-way ANOVA: $F_{2,53}$= 2.4; P= 0.10)

Table 4. Summary of population changes between site surveys of Arnside Knott (not including Heathwaite), Eaves Wood and Gait Barrows. 'Change %' relates to previous survey; 'Mean Density' refers to all active nests (no mound and active mound); 'Mean Volume' refers to active mound nests only. 1999 Arnside Knott and Eaves Wood data from Robinson (2001); 1986-2006 Gait Barrows data from Robinson and Robinson (2008).

Site Survey	**Nests Found**		**Active Nests**		**Mean Density**			**Mean Volume**		
	N	Change %	N	Change %	Within 100m	SD	Change %	m^3	SD	Change %
Arnside Knott										
1999	–	–	70	–	–	–	–	–	–	–
April 2009	68	–	57	-18.6	10.0	4.8	–	0.157	0.221	–
July 2016	39	-42.6	35	-38.6	10.2	5.8	+2.3	0.076	0.225	-51.6
June 2019	54	+38.5	45	+28.6	12.1	6.0	+18.6	0.043	0.073	-43.5
Eaves Wood										
1999	–	–	34	–	–	–	–	–	–	–
April 2008	42	–	37	+8.8	5	2.0	–	2.280	3.118	–
July 2019	7	-83.3	2	-94.6	1	0.0	-79.9	0.043	0.043	-98.1
Gait Barrows										
1986	–	–	83	–	–	–	–	–	–	–
1991	140	–	102	+22.9	–	–	–	–	–	–
1996	137	-2.1	112	+9.8	–	–	–	–	–	–
2006	169	+23.4	99	-11.6	–	–	–	–	–	–
April 2016	87	-48.5	47	-52.5	9.4	5.1	–	0.288	0.710	–
July 2019	86	-1.1	73	+55.3	19.9	9.0	+110.8	0.054	0.107	-81.3

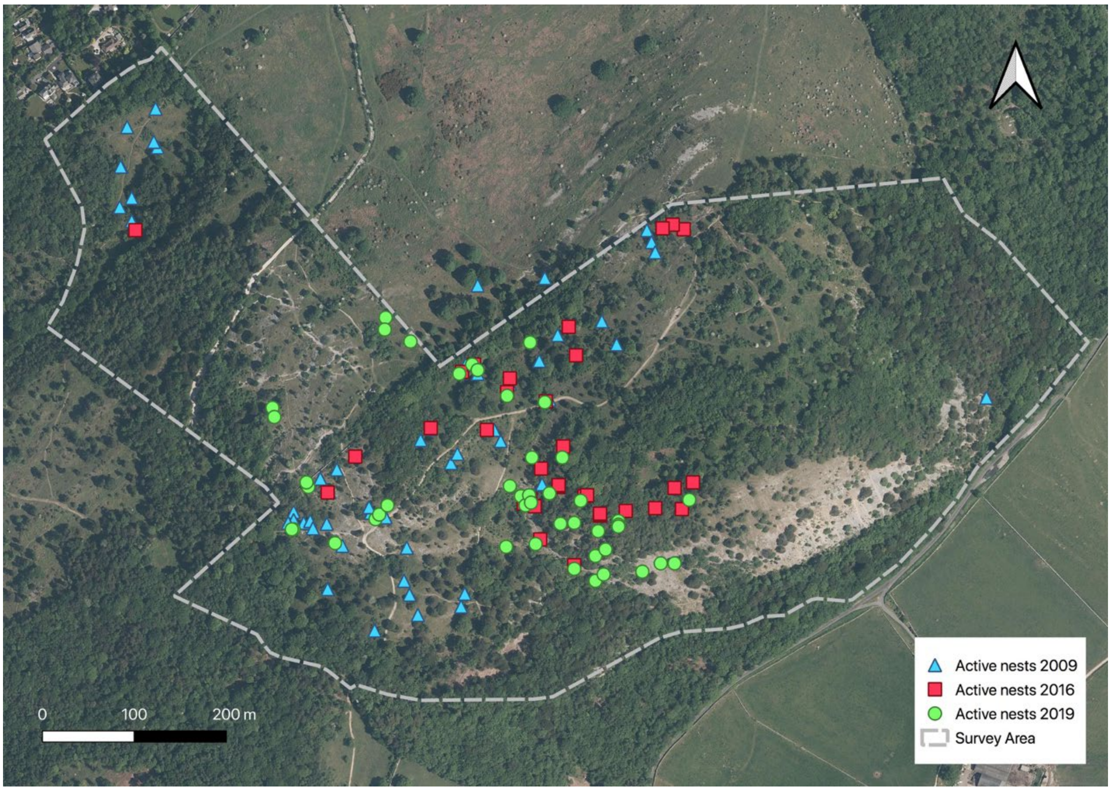

Fig. 6. Changes within Arnside Knott population between 2009-2019.

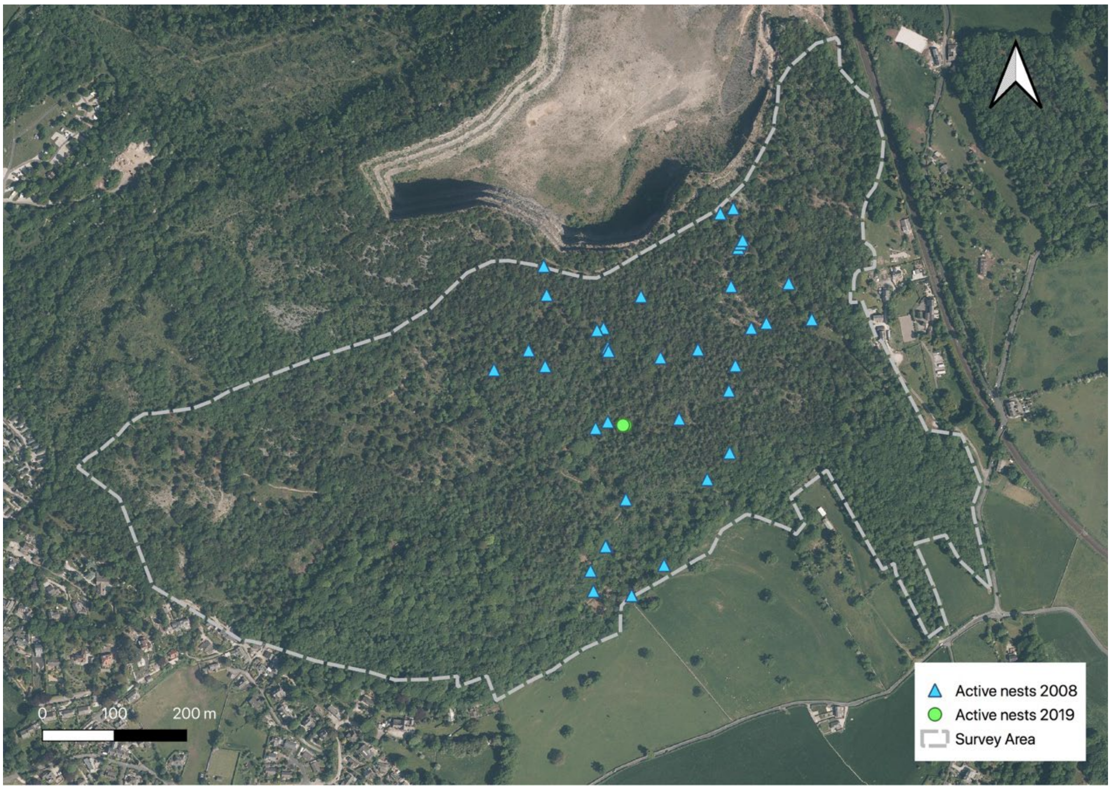

Fig. 7. Changes within Eaves Wood population between 2008-2019.

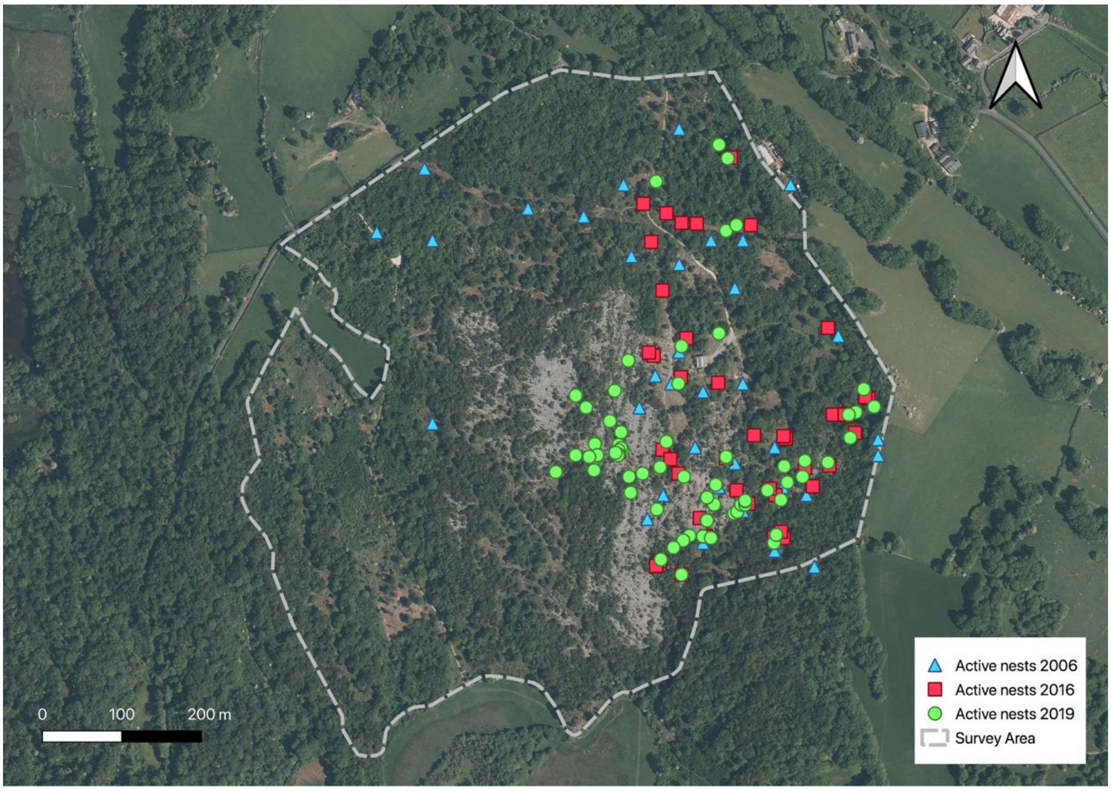

Fig. 8. Changes within Gait Barrows population between 2006-2019. Nest locations shown for 2006 are those given in Robinson & Robinson (2008) and do not represent the total number of active nests present at the time of the 2006 survey due to incomplete data.

3.5. Changes to the northern distribution

Table 4 summarises the compiled survey data from the three sites. The Arnside Knott population was reported to consist of 70 active nests according to a 1999 survey (Robinson, 2001). This decreased to 57 in 2009 (-18.6%) before a further decrease to 35 in 2016 (-42.6%). The 2019 survey located 45 active nests representing a change of +28.6% compared to the 2016 survey. Mean nest volume also decreased from 0.157m^3 (SD 0.221) in 2009 to 0.076m^3 (SD 0.225) in 2016 (-51.6%) before further decreasing to 0.042 m^3 (SD 0.072) in 2019 (-43.5%). However, mean nest density increased slightly from 10 (SD 4.8) in 2009 to 10.2 (SD 5.8) in 2016 (+2.3%) before furthering increasing to 12.1 (SD 6.0) in 2019 (+18.6%).

The Eaves Wood population consisted of 34 active nests according to a 1999 survey (Robinson, 2001) increasing to 37 in 2008 (+8.8%). Only 2 active nests were found in 2019 representing a change of -83.3%. Mean nest density decreased from 5 (SD 2.0) in 2008 to 1 (SD 0.0) in 2019. Mean nest volume also decreased from 2.280 m^3 (SD 3.118) in 2008 to 0.043 (SD 0.043) in 2019.

The Gait Barrows population consisted of 83 active nests were found in 1986; 102 in 1991 (+22.9%); 112 in 1996 (+9.8%) and 99 in 2006 (-11.6%) (Robinson & Robinson, 2008). A 2016 survey recorded 47 active nests, representing a -52.5% change from 2006. The

2019 survey located 73 active nests, constituting a change of +55.3% from 2016. Mean nest density also increased from 9.4 (SD 5.1) in 2016 to 19.9 (SD 9.0) in 2019 (+110.8%). Mean nest volume decreased from 0.288m^3 in 2016 to 0.054m^3 in 2019 (-81.3%).

4. Discussion

4.1. What is the current distribution of F. rufa within three key sites at the species' northern limit in Britain?

The results demonstrate a marked difference in the current distribution of *F. rufa* between the three survey sites (Table 2). Most notably, the Eaves Wood population appears to be very weak with only two active nests found. At the time of the survey, neither of these nests seemed to be strong with one appearing to be the remnants of a previously large colony beneath a mature Scots Pine *Pinus sylvestris* situated at the northern edge of a small glade, and the other being a small nest only several metres away near the centre of the glade appearing to have budded from the first but with few workers present. This is in stark contrast with both the Arnside Knott with Heathwaite and Gait Barrows populations, which were found to consist of a comparable number of active nests at 62 and 73, respectively.

Although Arnside Knott and Heathwaite are adjoined, there is a considerable separation between the nearest nests of Heathwaite and those on the western slopes of Arnside Knott itself to the northeast (approximately 650m). The Heathwaite nests are concentrated to the west, mostly around the wooded edges of open grassland on south/southwest facing slopes with trees to the north and east. Despite seemingly similar habitat existing across the central and southern sections of Heathwaite, *F. rufa* appears to be absent from these areas.

The nests found on and around Arnside Knott were found to occur most frequently in areas with an aspect between south and west, with the majority occurring on or just above the scree slopes leading up to the highest part of the site with mixed woodland cover. As the gradient of these slopes increases dramatically further to the east, it was not feasible to survey the full area so it is possible that more nests may be present than have been recorded. Notably, two of the largest nests on the site were located on the northside of Arnside Knott along with a third, heavily shaded nest in a thicket of young Hazel *Corylus avellana.* Each of the nests occurred within a 30m radius and were all linked by active trails of workers suggesting the three nests could represent a single polydomous colony (Maeder et al., 2016).

The Gait Barrows population exhibited a significantly higher active nest density than that of Arnside Knott with Heathwaite and a slightly higher overall mean nest volume, suggesting that the Gait Barrows population is currently the largest and strongest of the three sites. The majority of active nests were found to occur on and around the edges of the exposed limestone pavement that make up a significant area at the centre of the site. As observed by Robinson and Robinson (2008), nests appear to frequently utilise the increased topographic variation created after sections of pavement were removed or damaged through historic extraction work. Rides and glades in the woodland are also utilised along the eastern edge of the site.

Across all three sites, evidence was encountered of a strong preference for tree stumps, logs and raised stone as nest sites, which were often incorporated into mound structures. Activity centred around areas without a mound or thatch covering were fairly frequently encountered at both Arnside Knott and Gait Barrows but whether these were nests in the process of being established or declining nests was not always clear.

4.2. Does canopy cover influence nest distribution?

The overall results of canopy analysis indicate a relationship between canopy openness and nest status (Fig. 2.), namely between inactive nests and reduced canopy openness, supporting previous observations that shading contributes to nest decline and relocation in northern populations of *F. rufa* (Robinson, 2001). The results of separate within-site analysis, however, do not demonstrate consistent relationships across all sites. Despite the comparable mean canopy openness demonstrated by nest sites on Arnside Knott with Heathwaite (hereafter Arnside Knott) and Gait Barrows, the results showed a marked difference in the relationship between canopy cover and nest status between the two sites.

Arnside Knott canopy openness differed significantly between all nest types (Fig. 4. A), consistent with the overall findings that inactive nests were significantly more shaded than active nests. Furthermore, ‘active mound’ nests were significantly more shaded than those in the ‘active without mound’ category, the latter of which were primarily located on tree stumps and areas of expose limestone with little to no thatch present, often resulting in ambiguity between nests in the process of establishment and those in decline. Consequently, the significant difference in canopy openness between the two active nest categories may have several explanations. That some of the nests in the ‘active without mound’ group were in the process of establishment is consistent with previous findings that newly established nests, being smaller, require increased insolation for thermoregulation (Sorvari, 2013; Chen & Robinson, 2014). That the same group includes declining nests in areas of increased openness is also consistent with the findings of Robinson and Robinson (2008) who observed that growth and longevity of *F. rufa* nests decreased in areas of reduced shelter from canopy cover. It is possible that in some instances, both explanations were applicable to the same nest in that establishment is attempted in an unsuitable location regarding conditions and fails to successfully develop into an active mound, something commonly observed previously (C. McCoy pers. comm., 2019; E. Robinson pers. comm. 2019).

In contrast to Arnside Knott, Gait Barrows canopy openness was surprisingly consistent between nest types with no significant difference shown (Fig. 4. B). As active and inactive nests occurred in areas of similar canopy openness, it appears that shading from canopy is not currently the main driver of nest decline and relocation within the Gait Barrows population. The reason for this inconsistency between sites is unclear but it could be linked to differences in habitat characteristics. The woodland of the central limestone pavement areas of Gait Barrows where many of the nests were found is fragmented due to the nature of the substrate and exhibits a much-reduced field layer compared to the sites' peripheral woodland areas and the conditions found on much of Arnside Knott. As tree growth is slower and vegetation more localised within the pavement areas, this could result in more stable canopy conditions for nests located there, potentially under which nest decline and relocation occur due to factors unrelated to excessive shading.

Despite the contrasting within-site results, 'active mound' nests occurred most frequently under canopy openness between 30-60% on both sites, supporting the notion of an optimum canopy openness range for established nests. Whilst the low number of nest sites found prevented meaningful within-site analysis, the nest sites located within Eaves Wood, where the population has severely declined, exhibited a mean canopy openness below this 30-60% range.

4.3. Is there a relationship between canopy cover and active nest mound size?

Overall, active nest mounds were found to be significantly larger in areas of low canopy openness compared to those in areas of high canopy openness, which is consistent with a study of *F. lugubris* populations in the north of England (Chen and Robinson, 2014).

In contrast, separate within-site analysis of both Arnside Knott and Gait Barrows did not reveal a significant difference between canopy openness groups of either site, despite nest volume generally being higher in areas of low canopy openness. Notably, a high outlier was present in the >50% canopy openness group of both sites, each representing a large nest occurring in an area of increased canopy openness, going against the general trend of decreasing nest size under increasing canopy openness. However, the lack of significant results from within-site analysis suggests the relationship indicated by the overall significant result is not strong.

These findings support previous observations by Robinson (2001) that the species' shows a preference for more open woodland edge habitat with limited evidence to suggest that nests can persist under closed canopy conditions as colonies appear to either decline and/or relocate in response to reduced canopy openness.

Coppicing represents the most significant factor affecting canopy conditions on the three sites, yet it remains unclear precisely how beneficial the practice is for wood ant populations, especially if carried out beyond the dispersal range of colonised areas. However, no evidence has been found to suggest that coppicing adversely affects *F. rufa* populations on these sites but further research and continued monitoring is necessary.

4.4. How has the northern distribution changed over time?

Comparing the 2019 survey results with historic records reveals contrasting population trends between sites (Table 4), despite consistent site management throughout the period between surveys; namely coppicing (C. McCoy & G. Swainson pers. comm., 2019), a practice that has been credited with contributing to the stability of northern populations through the prevention of canopy closure (Robinson, 2001; Robinson & Robinson, 2008).

The Gait Barrows population has been well studied historically and remained stable between 1986-2006, as documented by Robinson and Robinson (2008). A 2016 survey, seemingly the first since 2006 (for which full data was unavailable for this study), evidenced a significant decline in active nests (-52.5%). This corresponds with a decline in active nests (-38.6%) and mean active nest volume (-51.6%) within the Arnside Knott population during the overlapping period between 2009-2016.

Since the declines shown in the 2016 surveys, numbers of active nests on Arnside Knott and Gait Barrows have increased by 2019 (+28.6% and +55.3% respectively), showing some signs of recovery. However, mean nest volume has decreased significantly within both populations (Arnside Knott -43.5% and Gait Barrows -81.3% between 2016-2019) suggesting colonies are weaker despite the higher number of active nests present.

In 2019 the Eaves Wood population comprised only two active nests representing a change of -94.6% since 2008. As no surveys of Eaves Wood were conducted between 2008 and 2019, the sites' population status in 2016 is unknown but the corresponding declines demonstrated on the two other sites and the personal observations of National Trust rangers (C. McCoy pers. comm., 2019) suggest that the Eaves Wood population decline began prior to 2016.

The results of the 2019 survey show that nests are absent from several previously occupied outlying areas of Arnside Knott indicating a contraction of within-site distribution (Fig. 6), whilst mean active nest density has remained stable. Gait Barrows surveys also indicate a contraction of within-site distribution (Fig. 8.), but in contrast to Arnside Knott, there has been marked increase in mean active nest density namely between 2016-2019.

The observed increases mean active nest density and number of active nests at Gait Barrows could be partly attributed to differences in survey timings as the 2016 survey took place in April, typically before mating and colony budding activity occurs from May onwards

(Robinson & Robinson, 2008), whereas the 2019 survey took place in July after new nests have typically budded from nearby established nests. However, the increase in nests on and around the central limestone pavement areas appears to indicate a shift in the within-site range, nonetheless.

The most notable of all changes is the severe decline exhibited by the Eaves Wood population (Fig. 7). Despite mean nest volume being high in 2008 compared to the other sites around the same period, records show the Eaves Wood population has consistently had the lowest number of active nests of the three populations and exhibited the lowest mean active nest density. The low number of small nests recorded in Eaves Wood during 2008 compared to other site surveys could be explained by the timing of the survey, taking place in April prior to the typical period of colony budding activity. It is also possible that the reduced number of small nests could be indicative of lower nest establishment success as isolated colonies are known to be susceptible to inbreeding depression which can lead to reduced fitness and reproductive success (Pamilo et al., 2016). Furthermore, the low mean canopy openness exhibited by the nest sites found in 2019 could suggest a reduction in suitable habitat within the formerly colonised areas. Consequently, if suitable habitat did not exist within the species' limited dispersal range and canopy cover increased over established nests this may have contributed to the failure to relocate and multiply, despite other areas of the site still appearing to offer suitable conditions. Aerial images (Fig 6, 7 & 8) and personal on-site observations during surveys indicate that current canopy conditions in Eaves Wood are less open with a lower proportion of woodland edge habitat than the two other sites.

From the survey data, it appears that in many cases, areas of the sites that have consistently contained active nests throughout the periods between surveys coincide with access routes such as footpaths and vehicle access tracks. As these areas are kept clear to maintain access, conditions remain largely stable enabling some colonies to persist over long periods in the same location or nearby; consistent with the observations of Robinson and Robinson (2008).

With the possible exception of Eaves Wood, the ongoing destabilisation and notable decline in populations across the sites between the mid to late 2000s and 2016 are unlikely to have been directly related to canopy cover, as site management remained largely consistent prior to and throughout the period (C. McCoy & G. Swainson, pers. comm., 2019). The seemingly concurrent nature of the declines suggest that climatic factors may have contributed; Robinson (2001) attributed widespread simultaneous declines observed in south Cumbrian populations in the late 20th century to higher than average spring rainfall and, in south west Finland, increased overwinter temperatures have been found to weaken wood ant populations through increased activity during typically dormant periods (Sorvari et al., 2011). There may have been a number of interacting factors behind the decline, however the precise cause or causes are currently unclear.

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