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1 **Fine-scale variation in natural nitrogen isotope ratios of ants**

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17 *Formica kozlovi*, Formicini, trophic ecology, mineral soil, organic soil

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1 **Abstract**

2 Stable isotopes provide a powerful means of elucidating the trophic ecology of organisms.
3 Analyses of variation in the ratio of nitrogen isotopes ($\delta^{15}\text{N}$) can provide insights into the
4 trophic position of species with broad diets and the ability to occupy multiple positions in
5 food webs, such as ants. The most powerful studies compare subjects across various spatial
6 scales, but to do so, local variation in $\delta^{15}\text{N}$ baselines must be taken into account. To date a
7 wide variety of baseline calibration methods have been employed, leading some authors to
8 suggest that a standard approach is needed, and that the reality of environmental variation
9 necessitates that this should be at fine scales. In this study, we examine the fine-scale
10 variation in $\delta^{15}\text{N}$ value of colonies of the ant *Formica kozlovi* Dlussky (Hymenoptera:
11 Formicidae: Formicini) along a sloped transect in Mongolia, and compare these with values
12 for associated soils in an effort to shed further light on this issue. We find variation in ant
13 $\delta^{15}\text{N}$ to the order of one trophic level (ca. 3‰), over a distance of only 1 km. Ant $\delta^{15}\text{N}$ was
14 highly correlated with soil $\delta^{15}\text{N}$, and variation in mineral soil $\delta^{15}\text{N}$ explained ca. 81% of the
15 variation in ant $\delta^{15}\text{N}$. This study underlines the importance of local-scale baseline corrections
16 for isotopic studies, particularly in environments where baseline variation might be expected.
17 It further suggests that $\delta^{15}\text{N}$ of mineral soils may provide a suitable baseline for ecological
18 studies of terrestrial arthropods.
19

1 **Introduction**

2 Stable isotope analysis is a useful tool for studies of the trophic ecology of a wide range of
3 species (Gannes et al., 1997; Post, 2002; Crawford et al., 2008; Wolf et al., 2009; McHugh et
4 al., 2010) and has become increasingly employed in studies of terrestrial arthropods (e.g.,
5 Blüthgen et al., 2003; Rickers et al., 2006; Bourguignon et al., 2011). Isotope ratios of
6 organismic nitrogen can be used to estimate relative trophic position because lighter isotopes
7 are lost at a higher rate through metabolic processes, leading to the enrichment of the rare ^{15}N
8 isotope in organisms relative to their diet (Gannes et al., 1997; Robinson, 2001; Post, 2002).
9 Within habitats, analysis of the $^{15}\text{N}/^{14}\text{N}$ isotope ratio ($\delta^{15}\text{N}$) can help disentangle complex
10 food webs and elucidate cryptic feeding behaviour, whereas comparing the isotope ratios of
11 organisms across sites can provide insights into broad-scale ecological questions such as the
12 effects of habitat disturbance and changes in trophic ecology during invasions.

13 A caveat for the use of nitrogen isotope ratios to investigate broad-scale ecological
14 questions is the need for appropriate calibration. Nitrogen isotope ratios vary globally (Craine
15 et al., 2009) and over smaller scales (Bai et al., 2009; Woodcock et al., 2012) as a result of
16 natural processes and human influence (Stewart et al., 2002; Fielder et al., 2007; Bai et al.,
17 2009). Thus, for isotopic values from multiple sites to be comparable, this spatial variation
18 must be taken into account (Fielder et al., 2007; Woodcock et al., 2012), and this is usually
19 achieved via calibration using a suitable local baseline (Post, 2002). However, a recent study
20 (Woodcock et al., 2012) reported major inconsistencies between isotope studies in the
21 approach to baseline corrections: around half of 34 isotopic studies reviewed used a single
22 baseline over the entire sampling area or used no baseline at all, whereas the remainder used
23 multiple baselines within sites over scales varying from <1 m to 50 km. A further problem
24 with reliable calibrations is choice of a suitable calibration organism. Calibrations often rely
25 on plant material and/or other arthropods of known diet (Blüthgen et al., 2003; Tillberg et al.,
26 2007; Feldhaar et al., 2009), but it may not be clear in many cases which species are suitable,
27 and whether these species are available across all the sites of interest (e.g., Gibb &
28 Cunningham, 2011).

29 Ants are important components of many ecosystems (Hölldobler & Wilson, 2009) but
30 are also often cryptic in their behaviour and feeding preferences and can occupy a wide range
31 of trophic levels, posing challenges for interpreting the trophic ecology of ant ecosystems
32 (Tillberg et al., 2007; Feldhaar et al., 2009). Nitrogen isotope analyses have been increasingly
33 employed in ant studies over recent years (Feldhaar et al., 2009), and have proven useful for
34 elucidating broad-scale aspects of ant trophic ecology including dietary changes in invasive

1 species (Tillberg et al., 2007; Menke et al., 2010; Wilder et al., 2011), and the influences of
2 habitat modification (Gibb & Cunningham, 2011; Woodcock et al., 2013) and fire (Caut et al.,
3 2014). Ant studies have revealed not only the expected differences in $\delta^{15}\text{N}$ between
4 subfamilies with differing life-history traits, but also strong spatial (Fielder et al., 2007;
5 Woodcock et al., 2012; Pfeiffer et al., 2014) and temporal (Tillberg et al., 2007; Menke et al.,
6 2010; Caut et al., 2013) variation in isotopic ratios. This highlights the need for accurate
7 baseline calibrations in comparative studies of the trophic ecology of the Formicidae, but a
8 suitable standard approach for calibration is still the subject of some debate (Woodcock et al.,
9 2012).

10 In this study we provide a fine-scale analysis of the variation in soil isotope ratios and
11 that for *Formica* ants recorded from the same locality in Mongolia. We show that variation to
12 the order of one trophic level can be found over distances as low as 1 km on a sloped transect.
13 These findings highlight the need for fine-scale calibrations of isotope ratio data, particularly
14 in any location where baseline variation might be expected. We also suggest that mineral soils
15 may provide a suitable baseline at least in some environments.

16

17 **Materials and methods**

18 **Sample site**

19 Our sampling site was a north facing slope located 16 km north of Uyanga in central
20 Mongolia (46°36.4'N, 102°13.7'E). We sampled 11 sites over a distance of 1 km at 100-m
21 intervals, spanning a slope from a hilltop to valley bottom over a height difference of 225 m
22 (Table 1). Site positions were recorded with a global positioning system (GPS) (Garmin
23 Oregon 450, Garmin Ltd., Olathe, Kansas, USA; accuracy 5 m). This area consists of
24 moderate altitude (>2 000 m) hilly terrain of open grassland and clusters of forest comprised
25 of mixed pine and larch. Forest understorey in the sampling area was very sparse to absent.

26

27 **Sample collection**

28 *Soil*

29 At each of the 11 sites, soil samples of 100 g were taken at each of four depths comprising
30 one sample of the organic layer (-5-0 cm) and three mineral soil layers (mineral 1: 0-5 cm;
31 mineral 2: 5-15 cm, and mineral 3: 15-35 cm) where possible (i.e., when soil extended this
32 far) according to the German scheme of 'Bodenkundliche Kartieranleitung 4' (Soil Survey
33 Manual 4; Boden, 1994).

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Ants

Formica kozlovi (Dlussky, 1965) (Hymenoptera: Formicidae: Formicini) were sampled using an aspirator from the upper parts of nests by removing the surface of logs or lifting rocks within 10 m of soil sampling points and immediately transferred to 99% ethanol. Multiple individuals were taken from three separate locations in the sampling area. These are assumed here to constitute samples from separate colonies, though they may in some cases represent separate galleries or entrances to the same large nest.

Formica kozlovi was by far the most prevalent ant species in the area. This species suffers from a dearth of available biological information, but is likely similar in behaviour to other Palaearctic *Formica* species, which have mixed diets and are integral to forest ecosystem nutrient fluxes (Fielder et al., 2007; Domisch et al., 2009). For example, *Formica fusca* Linnaeus, the common European black ant, forages on small insects, extra-floral nectaries, and aphid honeydew, and has colonies of 500-2 000 workers and up to 15 queens (Collingwood, 1979; Seifert, 2007). Foraging range in *Formica* spp. of similar individual size, colony size, and habitat is in the order of 10 m, though this can extend to 100 m in large-colony species (Sorvari, 2009). Thus, values of ant $\delta^{15}\text{N}$ reported here are likely to be representative of the local area of the site sampled and be derived from a mixture of sources.

Isotope analyses

Preparation of soil samples for nitrogen isotope analyses

Samples were first air-dried, then sieved (<2 mm), and subsequently ground and oven-dried: organic soil was dried for 24 h at a constant 65 °C, the mineral soil at 105 °C. Samples of 30-40 mg of organic soil and 60-80 mg of mineral soil were then folded into tin capsules.

Preparation of ant samples for nitrogen isotope analysis

Ants were removed from alcohol within 1 month of collection and air dried at room temperature (ca. 25 °C) for several days before storing on salt (NaCl) for analysis. Gasters were removed because of possible contamination from gut contents (Blüthgen et al., 2003; Feldhaar et al., 2009), and thoraces and in some cases heads were used in analyses. Components of single individuals were used in each sample, comprising 0.4-0.9 mg and folded into tin capsules.

Measurement of isotopic ratios

1 Analysis of $^{15}\text{N}/^{14}\text{N}$ ratios for the ant samples was undertaken at Graduate School of Natural
2 Science and Technology, Okayama University, Japan, using a continuous-flow mass
3 spectrometer coupled with an elemental analyser (IsoPrime EA; GV Instruments, Manchester,
4 UK). All isotopic values were shown as common $\delta^{15}\text{N}$ notation, as per million deviations
5 relative to international standard atmospheric dinitrogen. One laboratory standard
6 (acetanilide; $\delta^{15}\text{N} = -0.9\text{‰}$ calibrated relative to an internal standard USGS-40, L-glutamic
7 acid; $\delta^{15}\text{N} = -4.5\text{‰}$) was used for every 10 samples during analysis. The analytical error
8 during the overall process of mass spectrometry was less than $\pm 0.2\text{‰}$. Replicate samples
9 were run for each nest where available (Table S1). Analysis of soil $\delta^{15}\text{N}$ was undertaken at
10 the Soil Science Laboratory of Leibniz University, Hannover, Germany, using a Vario
11 ISOTOPE Cube elemental analyser (Elementar, Hanau, Germany). A two-point calibration
12 was performed using caffeine and L-glutamic acid.

13

14 **Statistical analysis**

15 Statistical analyses were performed in R v. 3.0.2 (R Core Team, 2014). Models were initially
16 formulated including only factors identified a priori as of potential influence following
17 Forstmeier & Schielzeth (2011). These models were tested against models with the factor of
18 interest removed using likelihood ratio tests (anova) in R. Environmental factors included in
19 analyses were distance (distance from site 1), altitude, habitat (presence or absence of forest
20 at the site), and the interaction between distance and altitude. Soil data were initially analyzed
21 using a linear model (lm) applied to log + 3 transformed values of soil $\delta^{15}\text{N}$, with depth
22 profile and site as factors. Differences between soil profile subgroups were then tested using
23 Tukey HSD, and this was followed by an analysis of environmental factors on these
24 subgroups with lm. Ant data were analysed using a mixed-effects model (lmer from the lme4
25 package), with the initial model including all environmental factors and colony within site as
26 a nested random factor.

27

28 **Results**

29 The $\delta^{15}\text{N}$ values of the three mineral soil layers were substantially higher than those of the
30 organic layer at each site, whereas there was little difference among mineral soil layers (Table
31 S2). This pattern was analysed with a linear model in R, using log + 3 transformed soil $\delta^{15}\text{N}$
32 data with depth profile and site as factors. This analysis indicated that $\delta^{15}\text{N}$ differed between
33 depth profiles ($P < 0.001$) and varied between sites ($P < 0.001$). A Tukey post-hoc analysis

1 revealed that mineral soil samples did not differ from each other ($P > 0.78$ in all cases)
2 whereas all mineral soil samples were significantly different to those from the organic layer
3 ($P < 0.001$ in all cases). Subsequently, mineral soil samples were pooled to obtain a single
4 mean value for each site, and the influence of environmental variables was examined
5 separately for these pooled mineral soil data and the organic layer data. Analyses with lmer
6 indicated that mineral soil $\delta^{15}\text{N}$ increased with distance from site 1 ($P = 0.043$; Figure 1).
7 There was also a significant interaction between altitude and distance ($P = 0.015$), but no
8 influence of altitude ($P = 0.083$) or habitat ($P = 0.23$). There were no significant effects for
9 organic soil (distance: $P = 0.54$; altitude: $P = 0.56$; habitat: $P = 0.44$).

10 Ant $\delta^{15}\text{N}$ ranged from 3.0 to 8.6‰, with a standard deviation within colonies of 0.5‰
11 (Table S1). Figure 1 shows the comparative variation in soil and ant $\delta^{15}\text{N}$ over the sample
12 sites used. Analysis with lmer indicated that $\delta^{15}\text{N}$ in ants also increased with distance from
13 site 1 ($P = 0.021$), though in this case there was also a significant inverse relationship with
14 altitude ($P = 0.031$). As for soil data, there was a significant interaction between altitude and
15 distance ($P = 0.009$) and no influence of habitat ($P = 0.19$).

16 Mean values of base ant $\delta^{15}\text{N}$ data were then calculated for each site (across
17 individuals) and compared to soil $\delta^{15}\text{N}$ data using correlations. These analyses indicated that
18 mean ant $\delta^{15}\text{N}$ was highly correlated with the mean value for mineral soils (Pearson's: $r =$
19 0.897 , $P < 0.001$) and also, though to a lesser degree, with organic soil $\delta^{15}\text{N}$ ($r = 0.722$, $P =$
20 0.012 ; Figure 2). Subsequently, ant $\delta^{15}\text{N}$ values were corrected for site by subtracting mean
21 mineral soil $\delta^{15}\text{N}$ values from mean ant $\delta^{15}\text{N}$ values for the same site and tested against
22 environmental variables as above using lmer. In contrast to base values, soil-calibrated values
23 of ant $\delta^{15}\text{N}$ did not vary with any factor (distance: $P = 0.13$; altitude: $P = 0.20$; habitat: $P =$
24 0.37).

25

26 Discussion

27 Our findings indicate that isotopic $\delta^{15}\text{N}$ values for ants can vary over fine scales within sites,
28 and track local variation in the $\delta^{15}\text{N}$ of the environment. Given that enrichment in ^{15}N is
29 typically around 3‰ per trophic level for ants (Minagawa & Wada, 1984; Feldhaar et al.,
30 2009), this variation can equate to at least one trophic level over distances as small as 1 km.
31 Woodcock et al. (2012) found that $\delta^{15}\text{N}$ baselines varied over a trophic level within a few
32 square kilometres of tropical forest, and showed that local correction of $\delta^{15}\text{N}$ values (within
33 transects) provided a better measure than global corrections (per site) for estimating variation
34 in $\delta^{15}\text{N}$ of tropical ant communities. They proposed that for reliable calibration, a minimum

1 distance of 500 m between samples and calibration sites should be employed. We find that
2 even over distances as small as 500 m, $\delta^{15}\text{N}$ can vary by close to 2‰ in some environments.
3 Although our site was deliberately chosen for its potential to show variation, many factors
4 can contribute to baseline variation in $\delta^{15}\text{N}$ (Bai et al., 2009; Craine et al., 2009) and as such
5 our site is certainly not unique. These data add weight to arguments in support of the need for
6 local-scale calibration in studies of ant isotope ratios (Woodcock et al., 2012), particularly if
7 there is reason to expect background variation in isotope ratios of the environment.

8 We found a strong relationship between $\delta^{15}\text{N}$ values of ants and that of soils. Variation
9 in mineral soil $\delta^{15}\text{N}$ in particular could explain 81% of the $\delta^{15}\text{N}$ variation in ants, whereas this
10 was only 52% for organic soils. This supports a previous ant study which used soil for
11 calibration when common plant material or herbivore species were unavailable across all
12 sample sites, in which soil $\delta^{15}\text{N}$ explained ca. 90% of the variation in ant $\delta^{15}\text{N}$ (Gibb &
13 Cunningham, 2011). Our data suggest that mineral soils may provide a better baseline than
14 organic soils, as the organic soil layer is subject to higher local variation. The organic soil
15 layer is presumably more subject to microhabitat variation in factors that may influence $\delta^{15}\text{N}$
16 such as moisture content, plant composition, fungal associations, and animal impacts
17 (Robinson, 2001; Hobbie & Ouimette, 2009). Mineral soils may thus provide a more
18 consistent pattern of broader slope trends as much of this variation has been homogenised.
19 Ant $\delta^{15}\text{N}$ may show similar consistency to mineral soil $\delta^{15}\text{N}$ because many ants forage over
20 an area large enough to similarly average microhabitat variation. It is generally accepted that
21 soil $\delta^{15}\text{N}$ increases with depth (Robinson, 2001; Hobbie & Ouimette, 2009). However, our
22 findings suggest that mineral soil $\delta^{15}\text{N}$ is consistent over depths of 5-35 cm, at least in a
23 Mongolian steppe environment. This is consistent with previous studies which show variation
24 in soil $\delta^{15}\text{N}$ is mostly associated with the organic/mineral soil boundary (Scheu & Falca,
25 2000; Vervaet et al., 2002; Hobbie & Ouimette, 2009), though variation within the mineral
26 layer can still be significant (e.g., Vervaet et al., 2002). Thus local sampling of mineral soils
27 from intermediate depth (ca. 10 cm) may provide a suitable baseline for ant $\delta^{15}\text{N}$ studies.

28 Baseline $\delta^{15}\text{N}$ can vary with topography because of the influence this has on soil
29 moisture content, nutrient availability, and nitrogen cycling rate (Bai et al., 2009). Our sample
30 site was a hill slope spanning a 225-m difference in altitude, and soil and ant $\delta^{15}\text{N}$ increased
31 over the transect from hilltop to valley bottom. Negative relationships with altitude and soil
32 and plant $\delta^{15}\text{N}$ have been found in various environments (Craine & Lee, 2003; Huber et al.,
33 2007; Liu & Wang, 2010), and have also been demonstrated in $\delta^{15}\text{N}$ values of European ants,
34 though over a much broader scale than examined here (Fielder et al., 2007). In addition to

1 natural processes, there was also a possible influence of human impact at our site through
2 presence of cows via faeces, which were observed at the site, or trampling of soil (e.g.,
3 Schrama et al., 2013).

4 Ants use a variety of diets, making selection of a suitable baseline difficult,
5 particularly in broad-scale studies where plant or animal species may be inconsistent. We find
6 here that mineral soils may provide a reliable baseline permitting calibrations to be done at
7 fine scales. Given that soil and foliar $\delta^{15}\text{N}$ values are correlated in a variety of landscapes
8 (Bai et al., 2009; Craine et al., 2009; Wang et al., 2010), mineral soil $\delta^{15}\text{N}$ may provide a
9 useful baseline for studies of various ant species in a range of environments. This should be
10 confirmed via studies in different environments and with a different presumed basis (i.e.,
11 other than altitude) for the variation in background $\delta^{15}\text{N}$. Regardless of baseline material, our
12 data further underscore the importance of calibration at local scales (Woodcock et al., 2012),
13 especially in regions where variation in $\delta^{15}\text{N}$ can be expected.

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2

1 **Figure captions**

2 **Figure 1** Variation in soil and ant mean (\pm SE) $\delta^{15}\text{N}$ over the sloped sample transect. Altitude
3 of sites is indicated by black and white triangles for forested and grassland sites, respectively.
4 White squares indicate data from mineral soils, white circles from organic soils, and black
5 diamonds indicate data from ants. Diamonds have been slightly right-shifted over the x-axis
6 for clarity.

7

8 **Figure 2** Relationship between mean soil and ant $\delta^{15}\text{N}$ across sample sites. Organic soil data
9 are indicated by open circles and dashed line-of-best-fit, mineral soil data by black squares
10 and a solid line.

11

12 **Supporting Information**

13 Additional Supporting Information may be found in the online version of this article.

14 Table S1. Ant $\delta^{15}\text{N}$ values (‰) for all sample sites. Distance is the cumulative distance from
15 site 1. Up to three individuals were sampled from each colony at each site. Site mean is the
16 average of the colony means for each site, whereas overall mean is the average over all
17 individuals.

18

19 Table S2. Soil $\delta^{15}\text{N}$ values (‰) for all sample sites. Soil was sampled at the organic layer and
20 at three depths (where possible) for mineral soils. Mean values are given over all mineral soil
21 depths.

22

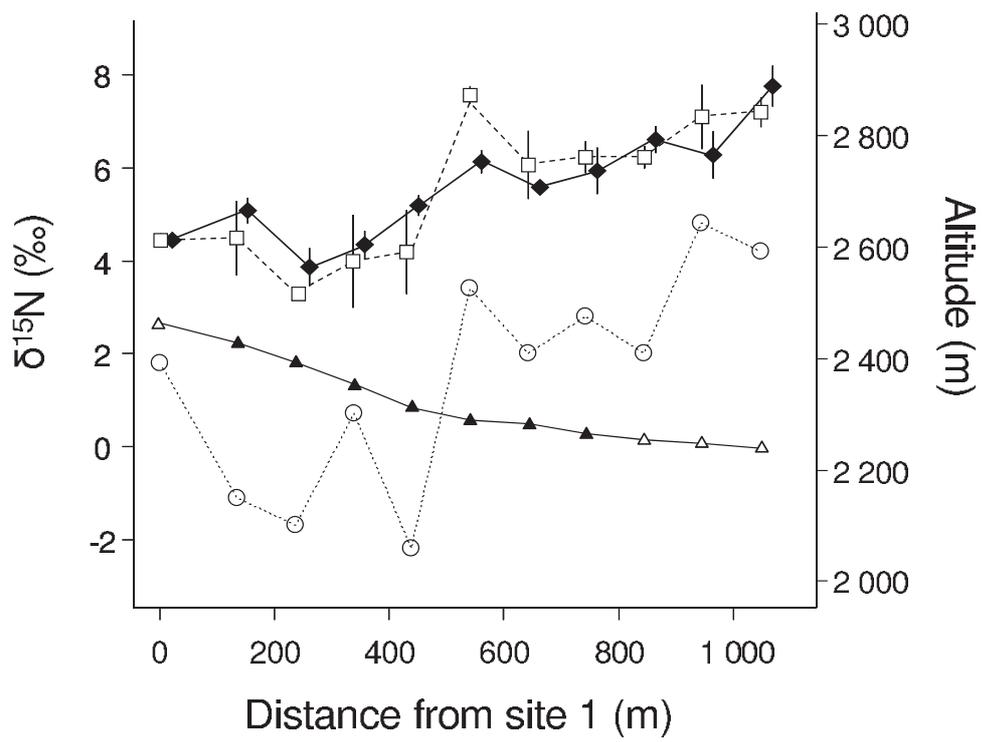
1 **Table 1** Global positioning system (GPS) coordinates (decimal degrees) and altitude of
 2 sampling sites, cumulative distance from site 1, composition of sites, and nesting material for
 3 ants

Site	Latitude	Longitude	Altitude (m)	Cumulative distance (m)	Site composition	Nesting material
1	46.59996	102.2314	2463	0	Grassland	Rocks
2	46.60111	102.2309	2428	135	Open Forest	Logs
3	46.60197	102.2305	2393	237	Open Forest	Logs
4	46.60289	102.2302	2353	340	Open Forest	Logs
5	46.60375	102.2299	2312	439	Open Forest	Logs
6	46.6046	102.2294	2289	540	Open Forest	Logs
7	46.60552	102.2292	2283	643	Open Forest	Logs
8	46.60642	102.2291	2265	743	Open Forest	Logs
9	46.60731	102.2288	2254	844	Grassland	Rocks
10	46.60821	102.2286	2247	945	Grassland	Rocks
11	46.60913	102.2284	2238	1049	Grassland	Rocks

4

5

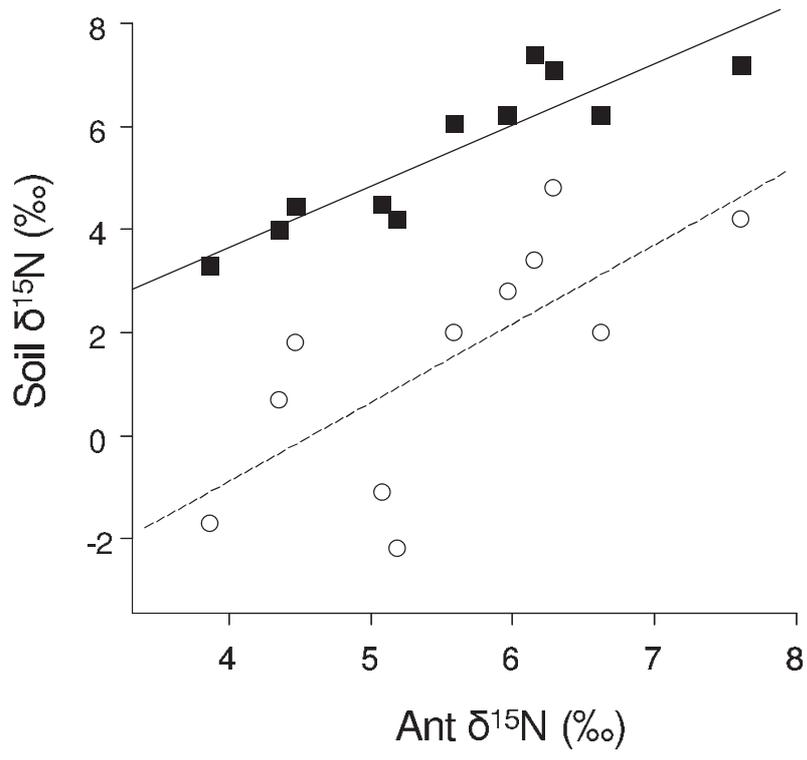
1 Figure 1



2

3

1 Figure 2



2

1 **Supporting Information**

2

3 Table S1. Ant $\delta^{15}\text{N}$ values (‰) for all sample sites. Distance is the cumulative distance from site 1. Up to three individuals were sampled from
 4 each colony at each site. Site mean is the average of the colony means for each site, whereas overall mean is the average over all individuals.

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Site	Altitude (m)	Distance (m)	Colony 1			Colony 2			Colony 3			Mean \pm SD				
			Individual 1	Individual 2	Individual 3	Individual 1	Individual 2	Individual 3	Individual 1	Individual 2	Individual 3	Colony 1	Colony 2	Colony 3	Site	Overall
1	2463	0	3.46	4.55	4.57	4.68	4.60	5.02	4.55	4.20		4.2 \pm 0.6	4.8 \pm 0.2	4.4 \pm 0.2	4.4 \pm 0.3	4.5 \pm 0.5
2	2428	135	4.74	4.09	5.58	5.21	5.59	5.30				4.8 \pm 0.7	5.4 \pm 0.2		5.1 \pm 0.4	5.1 \pm 0.6
3	2393	237	3.54	3.11	3.71	4.65	4.56	3.62				3.5 \pm 0.3	4.3 \pm 0.6		3.9 \pm 0.6	3.9 \pm 0.6
4	2353	340	5.18	5.10	3.00	3.70	3.36	4.31	6.81	3.79	3.87	4.4 \pm 1.2	3.8 \pm 0.5	4.8 \pm 1.7	4.3 \pm 0.5	4.3 \pm 1.2
5	2312	439	4.11	5.52	4.64	4.82	5.64	5.56	5.66	4.89	5.91	4.8 \pm 0.7	5.3 \pm 0.5	5.5 \pm 0.5	5.2 \pm 0.4	5.2 \pm 0.6
6	2289	540	6.32	5.48	6.27	6.39	5.48	5.46	6.95	6.93	6.00	6.0 \pm 0.5	5.8 \pm 0.5	6.6 \pm 0.5	6.1 \pm 0.4	6.1 \pm 0.6
7	2283	643	6.05	5.50	5.50	6.02	5.04	5.34	6.09	5.15		5.7 \pm 0.3	5.5 \pm 0.5	5.6 \pm 0.7	5.6 \pm 0.1	5.6 \pm 0.4
8	2265	743	5.55	5.40	5.37				6.43	6.45	6.48	5.4 \pm 0.1		6.5 \pm 0.0	5.9 \pm 0.7	5.9 \pm 0.6
9	2254	844	6.38	6.15	6.64	7.32	6.96	7.34	6.02	6.66	6.13	6.4 \pm 0.2	7.2 \pm 0.2	6.3 \pm 0.3	6.6 \pm 0.5	6.6 \pm 0.5
10	2247	945	7.31	7.14	7.03	6.55	6.28	6.17	5.59	5.01	5.54	7.2 \pm 0.1	6.3 \pm 0.2	5.4 \pm 0.3	6.3 \pm 0.9	6.3 \pm 0.8
11	2238	1049	8.20	7.39	8.55	8.38			6.74	7.28	6.72	8.0 \pm 0.6	8.4	6.9 \pm 0.3	7.8 \pm 0.8	7.6 \pm 0.8

8

1 Table S2. Soil $\delta^{15}\text{N}$ values (‰) for all sample sites. Soil was sampled at the organic layer and at three depths (where possible) for mineral soils.

2 Mean values are given over all mineral soil depths.

3

Site	Altitude (m)	Distance (m)	Organic	Mineral			Mean \pm SD
			(-5-0 cm deep)	1 (0-5 cm deep)	2 (5-15 cm deep)	3 (15-35 cm deep)	
1	2463	0	1.8	4.3	4.6		4.5 \pm 0.2
2	2428	135	-1.1	2.9	5.3	5.3	4.5 \pm 1.4
3	2393	237	-1.7	3.2	3.4		3.3 \pm 0.1
4	2353	340	0.7	3	5		4.0 \pm 1.4
5	2312	439	-2.2	3.3	5.1		4.2 \pm 1.3
6	2289	540	3.4	7.7	7.2	7.8	7.6 \pm 0.3
7	2283	643	2	4.6	6.8	6.8	6.1 \pm 1.3
8	2265	743	2.8	5.6	6.8	6.3	6.2 \pm 0.6
9	2254	844	2	5.9	6.1	6.7	6.2 \pm 0.4
10	2247	945	4.8	5.9	8.3	7.1	7.1 \pm 1.2
11	2238	1049	4.2	7.7	7.3	6.6	7.2 \pm 0.6

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