



Ant mounds extend the duration of plant phenology events and enhance flowering success

Rikke Reisner Hansen¹ · Knud Erik Nielsen¹ · David Bille Byriel² · Christian Damgaard¹ · Morten Tune Strandberg¹ · Inger Kappel Schmidt² · Joachim Offenberg¹

Received: 5 September 2022 / Accepted: 12 January 2023 / Published online: 7 February 2023
© The Author(s), under exclusive licence to Springer Nature B.V. 2023

Abstract

Mound-forming ants are important ecosystem engineers as they increase habitat heterogeneity, thus supporting multiple biotic interactions. How these ant-mediated changes in abiotic factors translate into temporal biotic heterogeneity, is a less studied subject. In a case study localized on a Danish heath, we investigated how ant mounds (mineral and organic mounds) affected the phenology and flowering success of five plant species growing on or between ant mounds (*Lasius flavus* and *Formica exsecta*). Specifically, we focused on the phenophases' stem elongation, flowering and seed set. All plant species showed significantly earlier phenophases on the mounds compared to control patches between mounds. These advances resulted in two distinct flowering seasons for one plant species and prolonged continuous flowering seasons for the four other species, when mound and non-mound plant seasons were combined. Likewise, stem elongation and seed set seasons were prolonged, with either two distinct seasons or one continuous season, depending on plant species. Two plant species exhibited increased survival up to the flowering stage when growing on ant mounds, since they flowered before a drought killed a large part of the population. Probable drivers behind these effects, as revealed by a structural equation model, were elevated surface temperature and other soil edaphic factors responsible for plant growth. Furthermore, the direct effects of the ant mounds were nearly twice as high for the organic mounds of *F. exsecta* compared with the mineral mounds of *L. flavus*. Possible implications are more resilient ecosystems, as prolonged seasons can mitigate phenological mismatches between interacting species.

Keywords Ant mounds · Biodiversity · Ecosystem engineering · Ecosystem resilience · Interactions · Phenological mismatch

Introduction

During nest building activities, mound-forming ants increase habitat heterogeneity in their environment. For example, they dig extensive underground channels and chambers whereby they redistribute soil nutrients. They pile up

collected soil and/or organic materials on the soil surface, which results in mound slopes with a reduced plant cover on active mounds. The exposed soil can catch and accumulate heat from solar radiation (Cammaraat and Risch 2008). This affects the physical and chemical properties of the soil and produces microclimatic gradients on and around ant mounds, with mounds often showing increased nutrient content, soil porosity and temperatures, as well as reduced water content (Kadochová and Frouz 2013; Véle and Holusa 2016). All of which is an alteration of the entire soil metabiome (Reverchon and Méndez-Bravo 2021). These abiotic changes modulate resources and environments for other organisms, which are reflected in the composition of associated biota that can differ substantially between mounds and their surroundings (Boots and Clipson 2013; Parmentier et al. 2014). On mounds, for example, there is excess aeration around roots, an elevated dry spot during floods. Elevated nutrient

Handling Editor: Miriama Malcicka.

✉ Rikke Reisner Hansen
rrh@ecos.au.dk

¹ Section for Plant and Insect Ecology, Department of Ecoscience, Aarhus University, C. F. Møllers Allé 4, 8000 Aarhus C, Denmark

² Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg, Denmark

contents and elevated temperatures may favor some plant species more than others and in this way affect plant species composition (Grime 1977; Frouz and Jílková 2008). Multiple studies have found significant effects of ant mounds on plant communities (King 1977; Folgarait et al. 2002; Dostál 2005; Lenoir 2009; Streitberger et al. 2017) and plant physiology (Dostál 2007; Ehrle et al. 2017; Farji-Brener and Werenkraut 2017; Hidalgo et al. 2021), with positive implications for other trophic levels (Li et al. 2018). Thus, it is clear that ant mounds provide both abiotic and biotic spatial heterogeneity. These effects coupled with the remarkable high biomass of ants (Gissel Nielsen et al. 1976) as well as the potential high density of ant mounds (Dostál et al. 2005; Steinmeyer et al. 2012) mean they can have considerable impact on ecosystem functioning (Farji-Brener and Werenkraut 2017; Wills and Landis 2018).

A much less studied aspect of ant mounds is how increased spatial heterogeneity in the abiotic environment may affect biotic temporal heterogeneity. For example, plants may respond to changes in the abiotic environment or the direct effect of ants by varying their growth rates, ultimately resulting in displaced or expanded plant phenology (Franks et al. 2007; Frei et al. 2014; Vilela et al. 2014). Small scale variations in temperature and moisture can alter flowering and other phenology events via phenotypic plasticity (Rathcke and Lacey 1985; Franks et al. 2007; Reverchon and Méndez-Bravo 2021). Likewise, soil potassium content (Dahlgren et al. 2007; Jan 2018) and topography (Jackson 1966) may affect phenology. Dahlgren et al. (2007) found that soil temperature, plant cover, slope and soil potassium content explained 83% of the variation in flowering time in an annual herb. Because ant mounds influence these measures, the phenology of plants growing on mounds is likely to be affected.

Changes in phenology patterns, e.g., due to climate change, may cause temporal mismatches between plants and their interacting arthropods, such as herbivores, pollinators and seed predators (Høye et al. 2007; Schmidt et al. 2016; Morton and Rafferty 2017). Furthermore, increased interspecific floral overlap may further induce-decreases in productivity and herbivory, and as such, affect both plant species reproduction and interacting species (Vilela et al. 2018). On the other hand, extended plant phenophases (extended periods of phenology events) induced by abiotic variation may mitigate such mismatches by increasing the temporal overlaps between plants and their interactors (de Manincor et al. 2020). Just as phenological mismatches can lead to loss of ecosystem functionality and weakening of interaction network structures (Burke et al. 2013; Forrest 2015), increased temporal overlaps between plants and their interacting arthropods strengthen functionality and stability. Among other things, resilience of an ecosystem to climate change or other perturbations depends on abiotic

variation and, thus, the distributions of phenological events (Encinas-Viso et al. 2012; Vilela et al. 2014; Beller et al. 2018). Increased season lengths can produce more stable networks with higher resilience (Encinas-Viso et al. 2012). If ant mounds affect plant phenophases in ways that lead to overall prolonged plant seasons, we argue that their presence may increase ecosystem resilience through a widening of the temporal window available for interactions. With this in mind, we find it relevant to quantify the potential effects of ant mounds upon plant phenology.

In this study, we specifically investigated whether plants associated to ant mounds responded to altered microclimatic and soil edaphic conditions on the mounds. We hypothesized that plants on mounds exhibit earlier phenophases compared to their counterparts growing between mounds. As different ant species construct different mound types (Wu et al. 2010) (i.e., some construct mounds made of organic materials and some build massive mineral mounds), we also hypothesized that this would lead to a further differentiation in phenology responses. We hypothesized that earlier development among mound plants can positively affect their flowering success under severe weather events, such as droughts.

Methods

Study area

The study was conducted at Sepstrup Hede, Denmark (coordinates: 56° 05' 04.9 N, 9° 25' 12.7 E), in 2018. The study area is an inland heath developed on outwash plains with sandy and nutrient-poor soils. The heathland is dominated by *Calluna vulgaris* with interspersed *Empetrum nigrum*, *Vaccinium uliginosum* and *Vaccinium vitis-idea*. Small patches with *Erica tetralix* are present in low-lying areas. The full extent of the heath covers an area of 418 ha, and the study area covers approximately 11 ha.

Ant species

We investigated the two mound-forming ant species *L. flavus* and *F. exsecta*, which are found in high abundances in Danish heathland ecosystems. Nest densities of both species vary, depending on management type and soil conditions and, thus, range from 0.01 nests per m² in unmanaged heathlands to 0.31 nests per m² in grazed heathlands for *L. flavus*. For *F. exsecta* densities range from 0.01 nests per m² in grazed heathlands to 0.03 nests per m² in unmanaged heathlands (Hansen et al. 2020). The studied heath was managed with grazing cattle. The mounds built by these two species differ in their physical properties. *L. flavus* mounds are built mainly of mineral soil and have a relatively stable vegetated surface (though with less vegetation compared to

non-mound surfaces), whereas the mounds of *F. exsecta* are mainly built of organic materials (e.g., seeds and straws) and with only sparse vegetation restricted to the peripheral boundary of the mound.

Plant phenology

With a paired design, we compared plant phenology on ant mounds with equal sized control heath patches (using 0.25 m² frames) situated > 1.5 m south of each mound and in an area not directly affected by other ants. One frame per patch type (mound, control) was surveyed. A pair of mound and control patches are henceforth referred to as a plot. In total, we selected 14 *L. flavus* and 17 *F. exsecta* mounds (all active) and their respective control patches. For all selected mounds, ants were identified in the field using a handheld magnifier (10×). All active mounds within the study area were inspected and the ones with a suitable control area (not affected by other ant colonies) were selected. As focal plant species, we selected those that we were able to find on both ant mounds and in control patches and with a minimum of five individuals per frame. For *L. flavus* we identified five species meeting these criteria (*Rumex acetocella*, *Luzula campestris*, *Hieracium pilosella*, *Galium saxatile* and *Festuca ovina*), and for *F. exsecta* we identified one species (*F. ovina*) (See online resource 1 for additional plant species information). In each frame, we randomly selected five individuals of each species for phenological assessments. Each plant individual was marked with differently colored plastic strips for recognition. For *L. flavus* mounds, we selected 70 individuals (14 mounds*5 plant individuals) of each plant species growing on ant mounds and 70 individuals growing in the control patches. For *F. exsecta* the numbers were 85 individuals on mounds (17 mounds*5 plant individuals) and 85 individuals off mounds.

Plant phenology was recorded for all individuals with a seven day interval in 2018 from April 3 to the June 28 following the extended BBCH scale for mono- and dicotyledonous plant species (Hess et al. 1997). This is a system developed for uniform coding of phenologically similar growth stages of mono- and dicotyledonous plant species, covering 10 principal growth stages and up to 10 secondary ones (online resource 2). From May until the beginning of August, Denmark experienced a severe drought, and many of the selected plants died during the study. We registered each mortality event to estimate survival rates. For statistical reasons, all registrations within a patch ended when there were less than three plant individuals left alive. After June 28, nearly all individuals had died as a result of the drought or completed lifecycle and, consequently, registration ended. Data have been uploaded to the Open Science Framework (Hansen 2020).

Using pin-point frames with the same dimensions as the patch frames (0.25 m²), we registered plant species composition of all plant species for both patch types. This was done in order to be able to quantify whether the selected plant species were more abundant on the mounds. A schematic drawing of the full sampling design, including abiotic measurements, can be found in supplementary materials (Online resource 3).

Abiotic measures

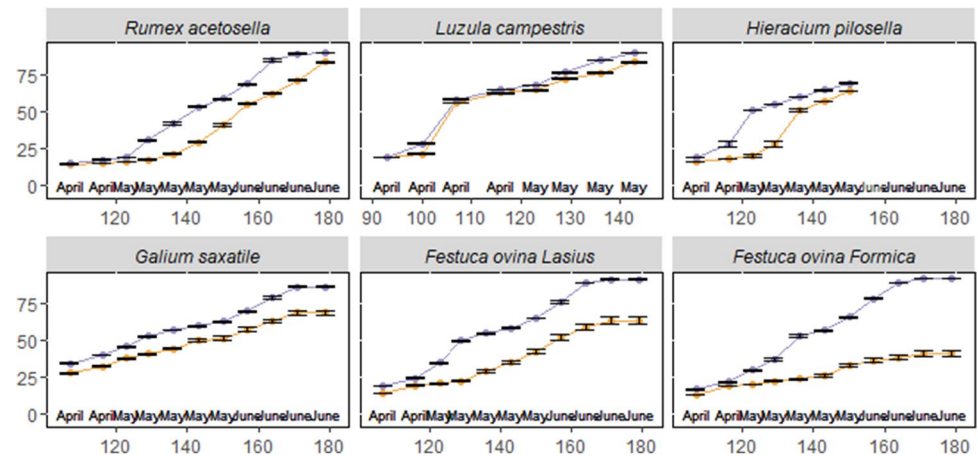
To assess abiotic properties, we measured temperature and moisture and took soil samples at three locations, which subsequently, were mixed to one sample for each patch type. Soil samples were later analyzed for pH, carbon, nitrogen, plant available phosphate, and potassium with the soil analysis protocols presented in online resource 4.

Surface temperatures on mounds and in control patches were registered at each sampling date using a thermal camera (FLIR C2) and the online software package 'FLIR tool' to extract data (2021 © Teledyne FLIR LLC). All registrations were done at noon and in similar sunny weather conditions. With the software package, we recorded the average temperatures within drawn circles covering the full extent of the mound and then overlaid on thermal photos of mounds and of control patches. We also registered in situ (top 5 cm.) soil moisture content (percent Volumetric Water Content—VWC) in four different positions on the mound (north, east, south, west) and, similarly, in the control patches at each sampling date. Soil moisture was measured with a moisture meter type HH2 with a theta probe ML3 (Delta-T Devices Ltd.). This device averages readings across the full length of the sampling depth. The samplings conducted at each plot were subsequently averaged over patch type prior to statistical analysis.

Statistics

Prior to modeling the response of phenology stage to patch type (mounds vs control patches), we plotted the recorded phenology stage for each individual plant and ant species averaged across plots for each week and calculated standard errors (Fig. 1). This to enable an estimation of the dispersion in data points and to see whether the phenology responses were linear. To test whether plant phenology was significantly affected by patch type, we employed linear mixed effect models via the function 'lme' in the R package 'nlme' (Pinheiro et al. 2022). All plant and ant species were tested in separate models, where sampling date/day of year (DOY) and patch type were included as fixed effects, and plant individuals nested within plot (pairs of mound and control patch) were assumed to be random effects. The temporal autocorrelation among repeated measures of the same individual

Fig. 1 Average phenology index values (BBCH scale) of mound and control plants by time (Day of year) on the x axis, plant species and ant species. Error bars represent standard errors. Purple lines represent plants growing on mounds and orange lines the control plants



was modelled using a continuous autoregressive structure of order one from the CAR1 function from the nlme package (Pinheiro et al. 2022). We included the interaction between DOY and patch type to test whether plant growth rate was significantly affected by mounds. Because the initial diagnostic plots showed signs of heteroscedasticity in the standardized residuals, we investigated residuals for each nominal predictor (i.e., DOY and patch type). These indicated that residuals varied independently across patch type. As such, we tested four different variance structures (varPower, varExs, varFixed and varIdent). For results on the modelled variance structures see online resource 5. Final model selection was determined through inspection of residuals as well as AIC values. As some plant individuals died during the experiment and this led to an unbalanced design, we used Type III anova with an F test for mixed effect models to extract F values. For this purpose, we used the function ‘anova.lme’ included in the ‘nlme’ package.

For the plant species with the most complete datasets (*F. ovina* on both mounds and *G. saxatile*), we constructed separate structural equation models (SEM) using the package ‘PiecewiseSem’ (Lefcheck 2016). This to further elucidate the direct and indirect effects of both patch type and abiotic variables on the advances in phenology stages. We constructed two types of SEM. One model per plant and ant species was constructed first to better distinguish between the direct and indirect effects upon phenology stages of each separate plant species. Subsequently, both ant species were modelled in the same Piecewise SEM for *F. ovina* to enable a comparison of the two different mound types. In both cases, we first built a linear mixed effect model including fixed factors, which for the separate plant species models consisted of patch type, soil moisture and surface temperature, as well as pH, potassium, Nitrogen, carbon and phosphate contents of the soil. For the model comparing mound types, fixed factors consisted of patch type only. As in the above linear models, we modelled the random term as plant individuals nested

within plot. Because the temporal autocorrelation arose due to repeated sampling (DOY), which was not included as a fixed factor in these models, we resolved the issue by including DOY in the random term as nested within plot and plant individual. For each model including the main fixed effects, we inspected residual variance across each nominal variable. Following this, the variable causing the heteroscedastic pattern was modelled via the above-mentioned variance structures. Final model selection was determined through a combination of inspection of residuals, Fishers C value and AIC values. The abiotic fixed factors were then added to the Piecewise SEM and modelled in a lme as a function of patch type. The random terms for the abiotic variables were adopted to match the applied sampling strategy, i.e., soil moisture and temperature were measured repeatedly and averaged across the full patch. Hence, the random term for these were DOY nested within plot. The remaining edaphic factors were sampled once and averaged across patches and, consequently, the random term merely included plot. Appropriate correlated errors were included, which were extracted from prior knowledge on the behavior of soil edaphic factors. For example, soil moisture is commonly negatively correlated with temperature (Al-Kayssi et al. 1990). For a full list of terms and full view of model structures, see online resource 6.

As plant phenophases differed between mounds and control patches, this could lead to temporal prolonged seasons of phenology stages when considering the total ecosystem holding both types of patches. To test this, we quantified the temporal range of three phenology stages by patch type and for each plant species. From the BBCH scale, we defined three life history events important for three biotic interactions: leaf herbivory, pollination and seed predation. These events were defined as stem elongation (stage 30–39), flowering (stage 60–67) and seed maturation (stage 80–89). To identify the starting and end dates of a particular event, we interpolated between the day preceding and the day

succeeding the corresponding index value. This was done with a cubic spline function to achieve a precise estimate of the exact date of each phenology event. From the interpolated means and their variation, we simulated a distribution for calculation of the 95% confidence intervals around the means of both onset and end of each phenology event. We then defined the mean extension of an event as the difference between end of the event in control patches and end of the event on ant mounds.

Flowering success was defined as the probability that a plant individual reached flowering stage. To estimate whether the percentage of plant individuals reaching the flowering stage differed between patch type, we performed a generalized linear mixed effect model with a binomial error distribution using the R package ‘lme4’ (Bates et al. 2015) with patch type and plant species as fixed effects and plot as a random factor. This was followed by a post hoc Tukey tests via the package ‘multcomp’ (Hothorn et al. 2008).

The hierarchical pin-point cover data for the five plants species were fitted to the beta-binomial distribution with two parameters, μ and δ , that measure the site mean cover and the degree of spatial aggregation, respectively (Damgaard and Irvine 2019). Statistical significance between patch type of the two parameters was tested using a loglikelihood ratio test in a linear mixed effect model with plot as a random factor.

Results

All plants, except for *L. campestris*, were significantly more advanced on the mounds (Table 1) compared to their control counterparts (Fig. 1; Table 1). In all cases, except for *L. campestris* and *H. pilosella*, the interaction between date and patch type was significant (Table 1), indicating that growth rates differed between patch types. For all species with a significant interaction, growth rates were higher on the mounds (reflected by the positive interaction terms in Table 1) and most pronounced for *F. ovina* (Fig. 1).

From Fig. 2, it is evident that life history events occurred earlier on ant mounds compared to control plots. For *F. ovina*, the presence of ant mounds resulted in two distinct flowering periods (on both types of ant mounds), as the early flowering on mounds was fully separated from the control plants growing between mounds (no overlap of confidence intervals). The other plant species showed overlapping flowering periods between mound and control plants, resulting in extended rather than distinct shifts in flowering seasons. Likewise, for the two other phenology stages assessed, there were two distinct stem elongation seasons for *H. pilosella* and *F. ovina* (on *L. flavus* mounds) and two distinct seed setting seasons for *R. acetosella*. The remaining plant species had extended seasons for these stages, except for *H. pilosella*

Table 1 Results of type III F test of linear mixed effects models testing the effects of patch type and sampling date (DOY) on the growth stages of five plant species

Plant species	Plots	Variable	F value	p value
<i>G. saxatile</i>	14	Intercept	81.9	<0.0001
		Patch type mound	20.9	<0.0001
		DOY	449.7	<0.0001
		Patch type mound*DOY	111.6	<0.0001
<i>H. pilosella</i>	11	Intercept	2852.7	<0.0001
		Patch type mound	73.5	<0.0001
		DOY	4356.9	<0.0001
		Patch type mound*DOY	145.4	0.29
<i>L. campestris</i>	14	Intercept	421.5	<0.0001
		Patch type mound	0.20	0.65
		DOY	934.06	<0.0001
		Patch type mound*DOY	1.4	0.23
<i>R. acetosella</i>	11	Intercept	17724.8	<0.0001
		Patch type mound	1315.2	<0.0001
		DOY	20101.5	<0.0001
		Patch type mound*DOY	180.5	<0.0001
<i>F. ovina Lasius</i>	14	Intercept	8.1	0.005
		Patch type mound	21.3	<0.0001
		DOY	23.1	<0.0001
		Patch type mound*DOY	39.5	<0.0001
<i>F. ovina Formica</i>	17	Intercept	201.5	<0.0001
		Patch type mound	1265.5	<0.0001
		DOY	204.7	<0.0001
		Patch type mound*DOY	2269.9	<0.0001

“Patch type mound” refers to the difference in phenology stages at the first sampling date compared to the control which is enclosed in the intercept

where neither control plants nor mound plants reached seed setting. For *G. saxatile* and *F. ovina*, all control plants died before seed set, leaving only the plants on the ant mounds with a completed life cycle (Fig. 2). The extended duration of seasons due to the presence of ant mounds is quantified for all plant species are presented in online resource 7. In conclusion, *L. campestris* was least affected by mounds, whereas and *F. ovina* were most affected.

The Piecewise SEM for *F. ovina* growing on *F. exsecta* mounds showed no significant pathway linking the presence of Formica mounds to the advanced phenology directly. There were, however, six indirect pathways that exerted significant effects. Temperature, potassium- and Nitrogen content all had significant positive effects on the phenology stages, and these were all significantly higher on the mound

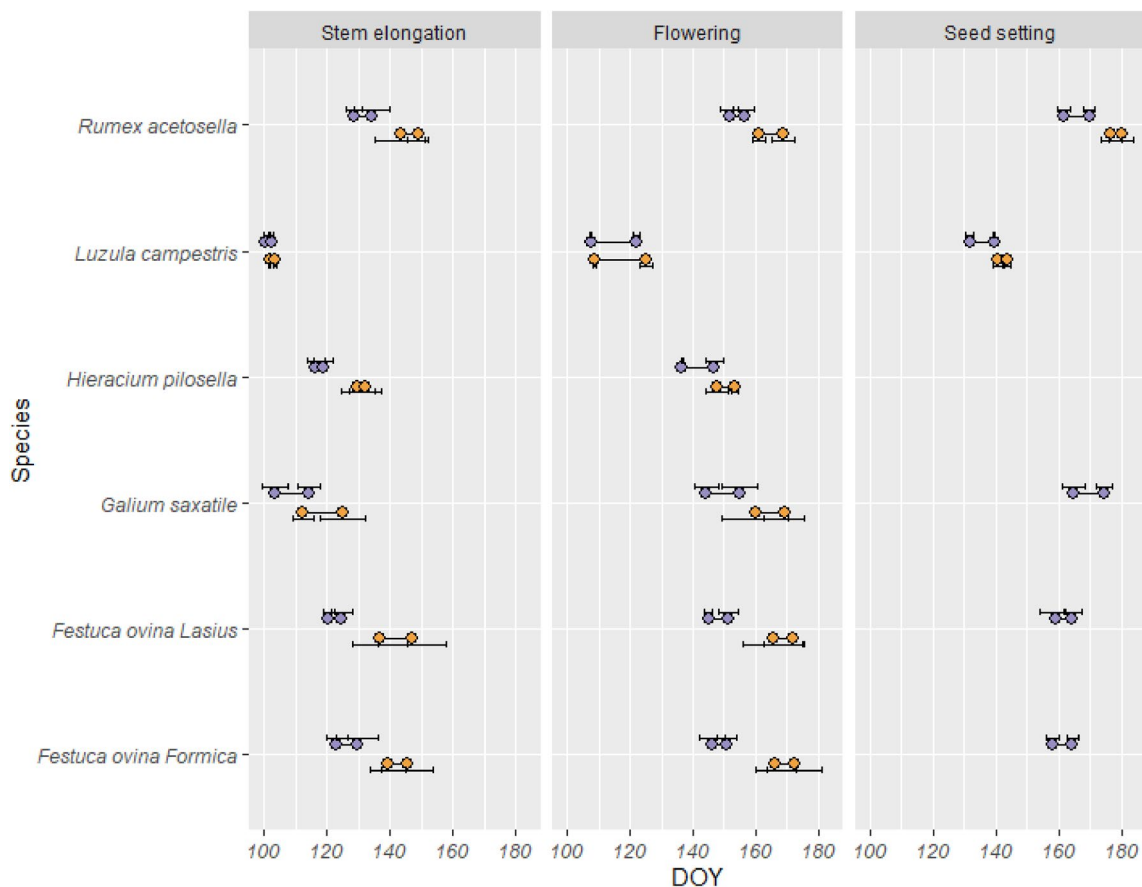


Fig. 2 The duration of three phenology events (stem elongation, flowering and seed setting) of plants growing on mounds and between mounds, by plant species. Symbols indicate the day a particular event was first recorded and the last day of the event, averaged across plots.

Colors indicate patch type with purple symbols showing mound plants and orange symbols showing control plants. Error bars represent the 95 percent confidence intervals of the means

than in the control patches. Soil moisture, Carbon content and pH were negatively correlated with phenology stage, where carbon content and pH were significantly higher on mounds and soil moisture significantly lower (Fig. 3a).

The *L. flavus* mounds also had significant effects upon the soil edaphic factors, with increased potassium, carbon, nitrogen and phosphate content, as well as increased temperature and pH. Soil moisture was significantly lower (Fig. 3a, b). The *F. ovina* plants growing on *L. flavus* mounds were positively impacted by direct effects of the mound, indicating that other factors than the recorded variables were responsible for the advanced phenophases on the mounds. In addition, there was a negative indirect effect of soil moisture and temperature on the phenology stage (Fig. 3b).

Phenology stages of *G. saxatile* also correlated positively with the direct effects of growing on top of a mound, the phenology of the plants was indirectly negatively impacted by soil moisture and surface temperature.

The model comparing the abiotic edaphic conditions between mound types revealed significant differences

between the mineral *Lasius* mounds and the organic *Formica* mounds (Fig. 4). Conditions on and within the organic mound type were generally drier, warmer and even more nutrient rich than on and within the mineral mound types (see Fig. 4 for coefficients). Furthermore, for this model the correlation with soil edaphic variables seen in the model for *F. exsecta* growing *F. ovina* plants was masked by a strong direct and significantly positive correlation between phenology stage and mound presence. This correlation was more than twice as high as the direct effects of *L. flavus* mounds (Fig. 4).

For two of the five plant species, there were a significantly higher chance of surviving until the flowering stage when growing on an ant mound (Fig. 5). *F. ovina* growing on either type of ant mound had more than twice as high survival ($65.6\% \pm 4.8$ S.E and $64.6\% \pm 5.1$ S.E for *L. flavus* and *F. exsecta*, respectively) than their controls ($30.3\% \pm 2.7$ S.E and $30.8\% \pm 6.2$ S.E for *L. flavus* and *F. exsecta*, respectively) ($p = 0.0003$ and $p < 0.0001$, respectively), and *G. saxatile* had a 62% higher survival

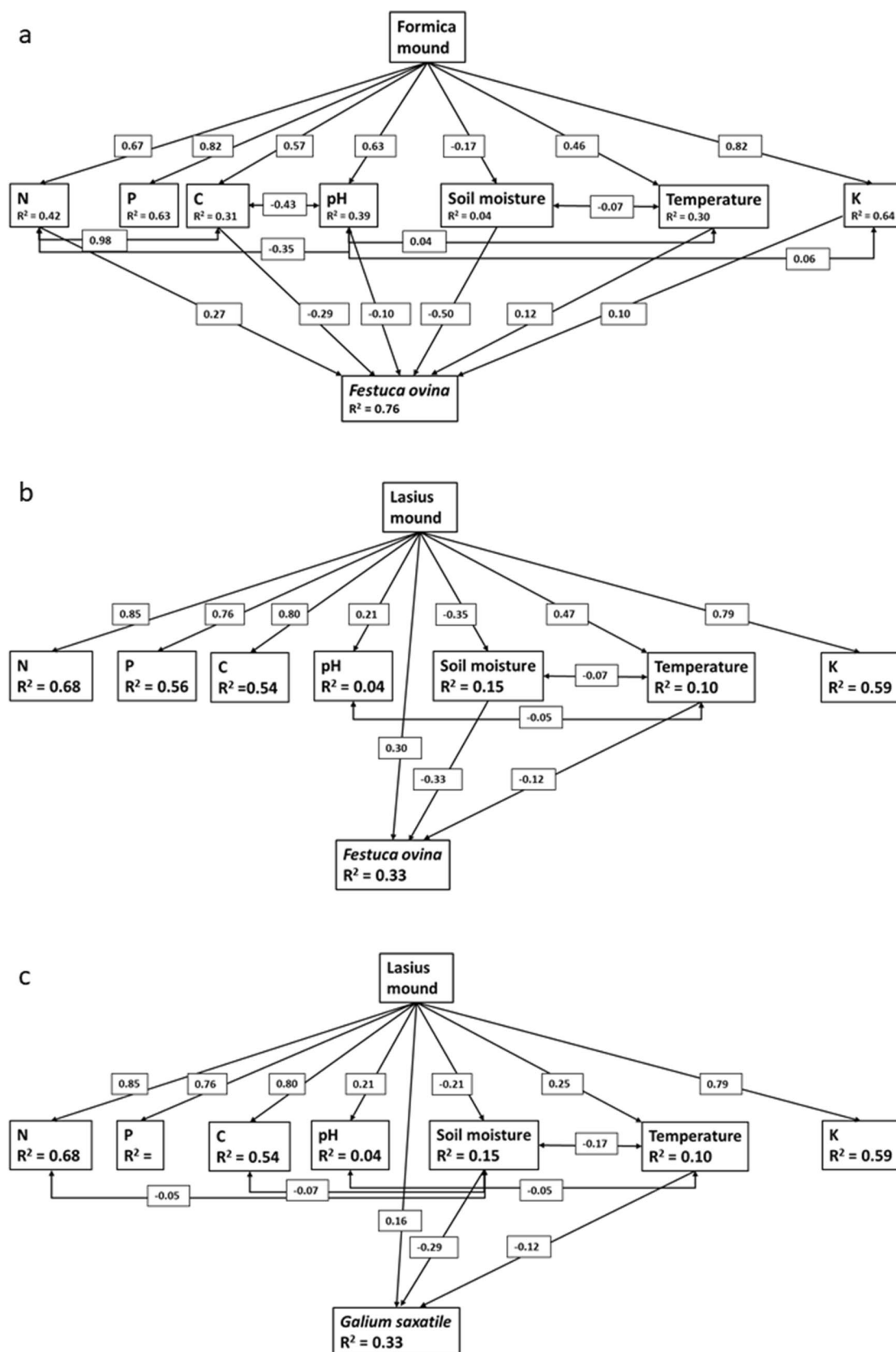


Fig. 3 Path diagram of Piecewise structural equation models for **a** *F. ovina* on *F. exsecta* mounds, **b** *F. ovina* on *L. flavus* mounds and **c** *G. saxatile* on *L. flavus* mounds. Effects are shown as standardized coefficients and only significant correlations are displayed

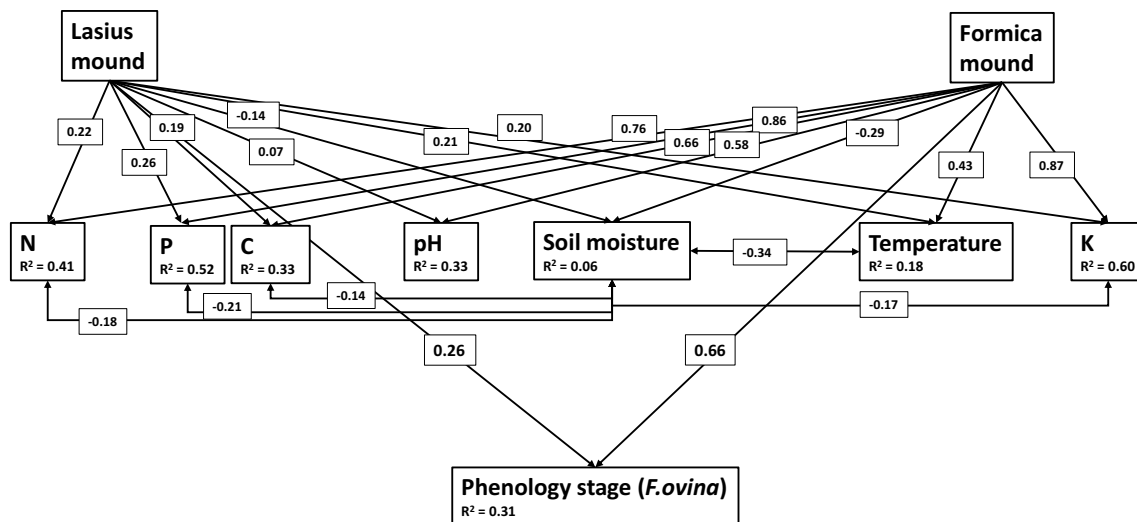
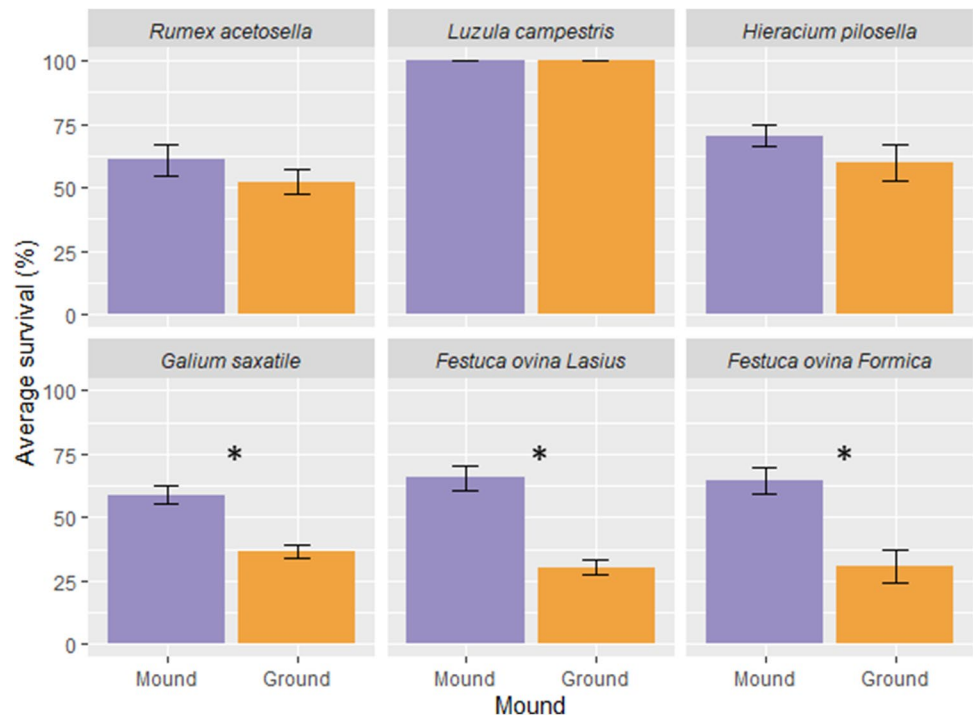


Fig. 4 Path diagram of Piecewise structural equation model for *F. ovina* plants as an effect of mound type. Effects are shown as standardized coefficients and only significant correlations are displayed

Fig. 5 The average percent of plant individuals that survive until the flowering stage. Error bars are standard errors and asterisks indicate significant ($p < 0.05$) differences between patch type. Purple columns represent mounds and orange columns the control patches



on mounds ($58.8\% \pm 3.7$ S.E) compared to its control plots ($36.4\% \pm 2.7$ S.E) ($p < 0.0001$). For the remaining three plant species, differences were not significant, although *R. acetosella* and *H. pilosella* showed similar trends.

G. saxatile and *R. acetosella* had a significantly higher coverage on the mounds compared to the control ($p = 0.01$ and 0.0001 respectively), whereas *H. pilosella* showed significantly higher coverage in the control patches ($p = 0.004$) (online resource 8).

Discussion

Here, we have demonstrated how mound-forming ant species cause different growth responses in plants. We detected significant effects of ant mounds on plant phenology for four out of five-plant species and on the mounds of both ant species. The ant mounds extended the overall plant flowering season by up to 22 days and, in some cases,

even caused two distinct seasons. These results are substantiated by previous studies showing that environmental heterogeneity begets intraspecific variation in flowering as well as root phenology (Dahlgren et al. 2007; Coogan et al. 2012). As the study year was particularly dry, we suggest that this mechanism may act as a buffer against future extreme climatic events (IPCC 2022). However, we cannot generalize for more typical years. Yet, we do foresee that the difference will be even more pronounced, due to the generally nutrient enriched and xerothermic environment (Frouz and Jílková 2008) produced by the mounds that will cause even greater contrasts in non-drought years.

In this study, we have shown that heterogeneity in the abiotic environment due to burrowing activities of ants can extend phenology seasons and counteract potential mismatches. Even at a local scale, we have shown that the steep abiotic gradients presented by the relatively small topographic change an ant mound causes can lead to two separate periods in phenological events. Diversification of phenological events holds many potential implications for interacting species. From the perspective of the plants, offset individuals may evade seed predators while still benefitting from mutualistic interactions, such as pollination (Vilela et al. 2014). This is because mutualists tend to favor early or peak flowering, whereas antagonists tend to favor peak seasons (Elzinga et al. 2007). From the consumers perspective, having separate seasons in the same plant species offers an extension of resource availability for both early and late interactions to occur simultaneously (Albrechtsen 2000; Encinas-Viso et al. 2012). As such, earlier seed set, flowering or stem elongation can accommodate multiple interactions, supporting both sap-feeding insects, pollinators and seed predators. Furthermore, it is likely that genetic diversity in interactor populations is supported, as both early and late genotypes will find available plant resources. How the extended phenophases affect these plant–insect interactions could be an interesting focal point for future studies.

Phenological phase extension was recently suggested as a means to manage phenological asynchrony in plant–animal mutualisms (Olliff-Yang et al. 2020). One of the techniques to accomplish this, was to increase local- to landscape scale heterogeneity. Following this argument combined with our findings, management benefitting mound formations is beneficial for overall phenological phase extension.

Albeit many studies indicate similar responses of interacting species to environmental change, phenological mismatch studies remain scarce, and most terrestrial studies focus on mutualistic interactions between plants and pollinators (Renner and Zohner 2018). However, in the present study, overlapping confidence intervals between patch types early in the season (Fig. 2) indicated that for some plant species, a complete decoupling of events does not become apparent until later in the season, while for other plant species, the

decoupling was evident during stem elongation, yet lessened towards flowering and seed setting. Because of this, the antagonistic interactions, such as with seed predating or herbivory, are affected differently than mutualistic interactions occurring earlier or during peak season. Hence, mismatch studies might benefit from the inclusion of multiple life stages of both plants and interacting species.

The varying responses among plant species may be explained by differences in individual plant growth strategies, resulting in differential phenotypic responses. For a plant like *L. campestris*, there was no effect of the mounds. This is a plant that flowers very early in the season before the differences in surface temperature and soil moisture take effect. The plant species with the most pronounced responses, both in terms of phenology and survival rate, were *F. ovina* and *G. saxatile*. The former is a highly drought-resistant perennial grass, with narrow folded leaves that reduce water loss. Furthermore, it is a species adapted to nutrient-poor heathland soils through its symbiosis with arbuscular mycorrhiza (Whittingham and Read 1982), a fungal partner which is promoted by the conditions inside ant mounds (Dauber et al. 2008). Hence, *F. ovina* can fully utilize the increased solar radiation and temperature on the mounds with limited ramifications due to physiological water stress. The warm and dry conditions on the ant mound acted as an inhibitor on the mineral mounds, but as a promotor on the organic mounds. This is likely explained by the way vegetation is distributed on the two mound types. Because vegetation on the organic mounds is limited to the perimeter, the plant roots are able to extend further and into a more stable environment than on the mineral mound. Here, the roots are subjected to higher variation in solar radiation and soil moisture as well as below-surface disturbances. There was also contrasting responses to the elevated potassium contents between the two mound types. Variation in potassium contents have strong effects on plant species composition (Roem and Berendse 2000) and regulates important plant physiological responses to water stress (Wang et al. 2013). This interaction with soil moisture is not reflected in the model. However, the positive response for the organic mound type for *F. ovina* may indicate that the plants can utilize potassium when extended towards a more stable environment. The obvious increased carbon content on the organic mounds inhibits plant growth in this model, whereas increased nitrogen content may help mediate this response. Nitrogen addition is a known accelerator of soil carbon turnover (Neff et al. 2002), which may add a likely explanation to the contrasting responses to carbon and nitrogen shown in the model (Fig. 3a). In previous studies, the distribution of *G. saxatile* has been found to be resilient towards increasing temperatures, lower soil moisture and prone to establishing in open canopy (Bässler et al. 2010). Large plasticity in flowering phenology, may allow them to

utilize increased temperatures and flower before soil moisture levels become lethal. Though *G. saxatile* was negatively impacted by increasing temperatures, the mound environment did have significant positive effects. Active mounds are generally open canopy environments with bare soil that provide beneficial habitats for the plants. Here, we also documented a higher coverage for *G. saxatile* on the mounds, adding support to this explanation. Hence, the ability of plants to respond with phenological plasticity may be a key selection trait towards the drier and warmer microclimate on the mounds in heathland soils. Due to these differential responses, the patchy distribution of ant mounds that increases overall ecosystem abiotic heterogeneity may be capable of altering both compositional and genetic variability in plant communities. Plants have been shown to respond positively to the enhanced nutrient levels with increases in reproduction traits, such as seed set (Wagner 1997) and root biomass (Dostál et al. 2005). As such, the mounds would not only increase survival in terms of advanced phenology, but they might also increase the plants' reproductive output. This remains an open question for future studies.

Not only were there contrasting responses between the indirect effects of the two mound types, but the structural equation model comparing mound types indicated a warmer environment with higher contents of plant nutrients in the organic mounds. As the model comparing mound types did not test the indirect effects of soil edaphic factors, the effects were converted into direct effects of mound type, which were more than twice as high for *F. exsecta* as they were for *L. flavus*. This discrepancy can be explained by various factors besides the higher nutrient content. The plant-arthropod interactions occurring on and in an ant mound are not limited to plant-ant interactions. For instance, most ant species tend aphids, which may have adverse effects on plant fitness. In nests of *L. flavus*, the most dominant root aphid is *Forda formicaria*, which feed of the sap of multiple graminoids, including *F. ovina* (Godske 1992; Depa and Wegierek 2011). This adds to the disturbance factor within *L. flavus* nests and may decrease fitness for the associated plants. *F. formicaria* is also tended by *F. exsecta*, however, as opposed to *L. flavus*, *F. exsecta* is mostly predatory and forages outside the nest (Seifert 2018). This behavioral trait combined with lower aphid abundances may decrease herbivory surrounding the ant nest and indirectly increase plant fitness.

In contrast to our study, a recent in situ study exploring the effects of precipitation and temperature on flowering phenology indicated that decreasing precipitation delayed flowering phenology (Rafferty et al. 2020). Another study has shown reduced survival and flowering of certain plant species as responses to increased drought conditions (Prieto et al. 2009). Potassium, in particular, is known to counteract negative effects of water stress (Jan 2018) because of the ability of potassium to regulate stomatal activity

and corresponding photosynthesis (Engels et al. 2012). Furthermore, a sufficient potassium content is essential for cell membrane stability and root elongation (Premachandra et al. 1990; Wang et al. 2013). As such, this extra level of complexity, coupled with the open canopy environment provided by ant mounds, may help explain the contrasting responses.

While this study concentrates on documenting the temporal heterogeneity, the spatial scale and spatial replication of the study was less of a focus point. However, as the mechanisms responsible for the phenological responses (abiotic gradients extending from the mounds) are similar within habitat types (Frouz and Jílková 2008), we argue that extrapolation of the study's results to other heathland sites is feasible.

Conclusion

Ant mounds are structures that are abiotically different from the surrounding heathland landscape. They differ both from the surrounding habitat and from each other interspecifically, as different species construct mounds differentially. This contributes to phenological variation of important heathland plants and their interacting species. Furthermore, they may ensure the completion of essential plant lifecycle events during extreme weather conditions. Thus, ant mounds play a potentially overlooked role in the shaping of biological processes. The decoupling of plant phenology events forms the potential for multiple interaction types occurring simultaneously over small spatial scales and increases the duration of available resources for both mutualists, commensalists and antagonists. In addition, where ant mound density is high, these effects may play a crucial role in eco-evolutionary terms.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11829-023-09946-z>.

Acknowledgements We would sincerely like to thank Lise Lauridsen for running soil sample analysis.

Author contributions KEN, JO, MTS and RRH conceived the ideas and wrote the methodology; RRH and KEN collected the data. IKS and DBB provided the soil sample analyses. RRH and CFD analyzed the data and RRH lead the writing of the paper. All authors contributed critically to the drafts and gave final approval for publication.

Funding This study was funded by the Aage V. Jensen Nature foundation.

Data availability Data is made available through the Open Science Framework (OSF). Hansen, R. R. 2020. Ant mounds extend the duration of plant phenology events and flowering success. Open Science Framework.

Declarations

Conflict of interest All authors declare that there are no conflict of interest.

References

- Albrechtsen RB (2000) Flowering phenology and seed predation by a tephritid fly: escape of seeds in time and space. *Écoscience* 7(4):433–438
- Al-Kayssi AW, Al-Karaghouli AA, Hasson AM, Beker SA (1990) Influence of soil moisture content on soil temperature and heat storage under greenhouse conditions. *J Agric Eng Res* 45:241–252
- Bässler C, Müller J, Dziock F (2010) Detection of climate-sensitive zones and identification of climate change indicators: a case study from the Bavarian Forest National Park. *Folia Geobot* 45:163–182
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. 67(1): 48
- Beller EE, Spotswood EN, Robinson AH, Anderson MG, Higgs ES, Hobbs RJ, Suding KN, Zavaleta ES, Grenier JL, Grossinger RM (2018) Building ecological resilience in highly modified landscapes. *Bioscience* 69(1):80–92
- Boots B, Clipson N (2013) Linking ecosystem modification by the yellow meadow ant (*Lasius flavus*) to microbial assemblages in different soil environments. *Eur J Soil Biol* 55:100–106
- Burkle LA, Marlin JC, Knight TM (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339(6127):1611–1615
- Cammeraat ELH, Risch AC (2008) The impact of ants on mineral soil properties and processes at different spatial scales. *J Appl Entomol* 132(4):285–294
- Coogan SCP, Nielsen SE, Stenhouse GB (2012) Spatial and temporal heterogeneity creates a brown tide in root phenology and nutrition. *ISRN Ecol* 2012:10
- Dahlgren JP, von Zeipel H, Ehrlén J (2007) Variation in vegetative and flowering phenology in a forest herb caused by environmental heterogeneity. *Am J Bot* 94(9):1570–1576
- Damgaard CF, Irvine KM (2019) Using the beta distribution to analyse plant cover data. *J Ecol* 107(6):2747–2759
- Dauber J, Niechoj R, Baltruschat H, Wolters V (2008) Soil engineering ants increase grass root arbuscular mycorrhizal colonization. *Biol Fertil Soils* 44(5):791–796
- de Manincor N, Hautekeete N, Piquot Y, Schatz B, Vanappelghem C, Massol F (2020) Does phenology explain plant–pollinator interactions at different latitudes? An assessment of its explanatory power in plant–hoverfly networks in French calcareous grasslands. *Oikos* 129:753–765. <https://doi.org/10.1111/oik.07259>
- Depa Ł, Wegierek P (2011) Aphid fauna of nests of *Lasius flavus*. *Aphids Other Hemipterous Insects* 17:73–79
- Dostál P (2005) Effect of three mound-building ant species on the formation of soil seed bank in mountain grassland. *Flora Morphol Distrib Funct Ecol Plants* 200(2):148–158
- Dostál P (2007) Population dynamics of annuals in perennial grassland controlled by ants and environmental stochasticity. *J Veg Sci* 18(1):91–102
- Dostál P, Březnová M, Kozlíčková V, Herben T, Kovář P (2005) Ant-induced soil modification and its effect on plant below-ground biomass. *Pedobiologia* 49(2):127–137
- Ehrle A, Andersen AN, Levick SR, Schumacher J, Trumbore SE, Michalzik B (2017) Yellow-meadow ant (*Lasius flavus*) mound development determines soil properties and growth responses of different plant functional types. *Eur J Soil Biol* 81:83–93
- Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G (2007) Time after time: flowering phenology and biotic interactions. *Trends Ecol Evol* 22(8):432–439
- Encinas-Viso F, Revilla TA, Etienne RS (2012) Phenology drives mutualistic network structure and diversity. *Ecol Lett* 15(3):198–208
- Engels C, Kirkby E, White P (2012) Yield and the source-sink relationships. In: Marschner H (ed) *Mineral nutrition of higher plants* (third edition). Academic Press, London, pp 85–133
- Farji-Brener AG, Werenkraut V (2017) The effects of ant nests on soil fertility and plant performance: a meta-analysis. *J Anim Ecol* 86(4):866–877
- Folgarait PJ, Perelman S, Gorosito N, Pizzio R, Fernández J (2002) Effects of *Camponotus punctulatus* ants on plant community composition and soil properties across land-use histories. *Plant Ecol* 163(1):1–13
- Forrest JRK (2015) Plant–pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? *Oikos* 124(1):4–13
- Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc Natl Acad Sci USA* 104(4):1278–1282
- Frei E, Ghazoul J, Pluess A (2014) Plastic responses to elevated temperature in low and high elevation populations of three grassland species. *PLoS ONE* 9:e98677
- Frouz J, Jílková V (2008) The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecol News* 11:191–199
- Gissel Nielsen M, Skyberg N, Winther L (1976) Studies on *Lasius flavus* F. (Hymenoptera, Formicidae): I. Population density, biomass, and distribution of nests. *Ent Meddr* 44:65–75
- Godske L (1992) Aphids in nests of *Lasius flavus* F. in Denmark, 1: faunistic description (aphidoidea, anoeciidae and pemphigidae; hymenoptera, formicidae). *Entomol Medd* 59(3):6
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111(982):1169–1194
- Hansen RR (2020) Ant mounds extend the duration of plant phenology events and flowering success. Open Science Framework. <https://doi.org/10.17605/OSF.IO/7KZ2M>
- Hansen RR, Nielsen KE, Offenberg J, Damgaard C, Byriel DB, Schmidt IK, Sørensen PB, Kjær C, Strandberg MT (2020) Implications of heathland management for ant species composition and diversity—is heathland management causing biotic homogenization? *Biol Conserv* 242:108422
- Hess M, Barralis G, Bleiholder H, Burh L, Eggers T, Hack H, Stauss R (1997) Use of the extended BBCH scale—general for the descriptions of the growth stages of mono; and dicotyledonous weed species. *Weed Res* 37(6):433–441
- Hidalgo FJ, Canepuccia AD, Arcusa J, Fanjul E, Álvarez G, Iribarne OO (2021) Black fire ant mounds modify soil properties and enhanced plant growth in a salt marsh in Argentina. *Estuar Coast Shelf Sci* 261:107534
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biomet J* 50(3):346–363
- Høye TT, Post E, Meltøfte H, Schmidt NM, Forchhammer MC (2007) Rapid advancement of spring in the High Arctic. *Curr Biol* 17(12):R449–R451
- IPCC (2022) IPCC, 2022: climate change 2022: impacts, adaptation, and vulnerability. In: Pörtner H-O, Roberts MTDC, Poloczanska KMES et al (eds) *Contribution of Working Group II to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, New York
- Jackson MT (1966) Effects of microclimate on spring flowering phenology. *Ecology* 47(3):407–415

- Jan MF (2018) Response of phenology, growth and productivity of maize hybrids to integrated potassium management. *Pak J Agric Res* 31:306–312
- Kadochová S, Frouz J (2013) Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (*Formica rufa* group). *F1000Research* 2:280–280
- King TJ (1977) The plant ecology of ant-hills in calcareous grasslands: I. Patterns of species in relation to ant-hills in Southern England. *J Ecol* 65(1):235–256
- Lefcheck JS (2016) piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol Evol* 7(5):573–579
- Lenoir L (2009) Effects of ants on plant diversity in semi-natural grasslands. *Arthropod-Plant Interact* 3(3):163–172
- Li X, Zhong Z, Sanders D, Smit C, Wang D, Nummi P, Zhu Y, Wang L, Zhu H, Hassan N (2018) Reciprocal facilitation between large herbivores and ants in a semi-arid grassland. *Proc R Soc B Biol Sci* 285(1888):20181665
- Morton EM, Rafferty NE (2017) Plant-pollinator interactions under climate change: the use of spatial and temporal transplants. *Appl Plant Sci* 5(6):1600133
- Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* 419(6910):915–917
- Olliff-Yang RL, Gardali T, Ackerly DD (2020) Mismatch managed? Phenological phase extension as a strategy to manage phenological asynchrony in plant–animal mutualisms. *Restor Ecol* 28(3):498–505
- Parmentier T, Dekoninck W, Wenseleers T (2014) A highly diverse microcosm in a hostile world: a review on the associates of red wood ants (*Formica rufa* group). *Insectes Soc* 61(3):229–237
- Pinheiro J, Bates D, R Core Team (2022) nlme: linear and nonlinear mixed effects models. R. C. Team: R package
- Premachandra GS, Saneoka H, Fujita K, Ogata S (1990) Cell membrane stability and leaf water relations as affected by phosphorus nutrition under water stress in maize. *Soil Sci Plant Nutr* 36(4):661–666
- Prieto P, Peñuelas J, Niinemets Ü, Ogaya R, Schmidt IK, Beier C, Tietema A, Sowerby A, Emmett BA, Lång EK, Kröel-Dulay G, Lhotsky B, Cesaraccio C, Pellizzaro G, De Dato G, Sirca C, Estiarte M (2009) Changes in the onset of spring growth in shrubland species in response to experimental warming along a north–south gradient in Europe. *Glob Ecol Biogeogr* 18(4):473–484
- Rafferty NE, Diez JM, Bertelsen CD (2020) Changing climate drives divergent and nonlinear shifts in flowering phenology across elevations. *Curr Biol* 30(3):432–441.e433
- Rathcke BJ, Lacey E (1985) Phenological patterns of terrestrial plants. *Annu Rev Ecol Syst* 16:179–214
- Renner SS, Zohner CM (2018) Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. *Annu Rev Ecol Syst* 49(1):165–182
- Reverchon F, Méndez-Bravo A (2021) Plant-mediated above- below-ground interactions: a phytobiome story. In: Del-Claro K, Torezan-Silingardi HM (eds) *Plant-animal interactions: source of biodiversity*. Springer International Publishing, Cham, pp 205–231
- Roem WJ, Berendse F (2000) Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities. *Biol Conserv* 92(2):151–161
- Schmidt NM, Mosbacher JB, Nielsen PS, Rasmussen C, Høye TT, Roslin T (2016) An ecological function in crisis? The temporal overlap between plant flowering and pollinator function shrinks as the Arctic warms. *Ecography* 39(12):1250–1252
- Seifert B (2018) *The ants of central and north Europe*, Lutra Verlags- und Vertriebsgesellschaft
- Steinmeyer C, Pennings PS, Foitzik S (2012) Multicolonial population structure and nestmate recognition in an extremely dense population of the European ant *Lasius flavus*. *Insectes Soc* 59(4):499–510
- Streitberger M, Schmidt C, Fartmann T (2017) Contrasting response of vascular plant and bryophyte species assemblages to a soil-disturbing ecosystem engineer in calcareous grasslands. *Ecol Eng* 99:391–399
- Véle A, Holusa J (2017). Microclimatic conditions of *Lasius flavus* ant mounds. *Int J Biometeorol* 61(5):957–961. <https://doi.org/10.1007/s00484-016-1275-z>
- Vilela AA, Torezan-Silingardi HM, Del-Claro K (2014) Conditional outcomes in ant–plant–herbivore interactions influenced by sequential flowering. *Flora Morphol Distrib Funct Ecol Plants* 209(7):359–366
- Vilela AA, Del Claro VTS, Torezan-Silingardi HM, Del-Claro K (2018) Climate changes affecting biotic interactions, phenology, and reproductive success in a savanna community over a 10-year period. *Arthropod-Plant Interact* 12(2):215–227
- Wagner D (1997) The influence of ant nests on Acacia seed production, herbivory and soil nutrients. *J Ecol* 85(1):83–93
- Wang M, Zheng Q, Shen Q, Guo S (2013) The critical role of potassium in plant stress response. *Int J Mol Sci* 14(4):7370–7390
- Whittingham J, Read D (1982) Vesicular-arbuscular mycorrhiza in natural vegetation systems. *New Phytol* 90: 277–284. <https://doi.org/10.1111/j.1469-8137.1982.tb03259.x>
- Wills BD, Landis DA (2018) The role of ants in north temperate grasslands: a review. *Oecologia* 186(2):323–338
- Wu H, Lu X, Wu D, Yin X (2010) Biogenic structures of two ant species *Formica sanguinea* and *Lasius flavus* altered soil C, N and P distribution in a meadow wetland of the Sanjiang Plain, China. *Appl Soil Ecol* 46(3):321–328

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.