

Parasitic plants indirectly regulate below-ground properties in grassland ecosystems

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Parasitic plants are one of the most ubiquitous groups of generalist parasites in both natural and managed ecosystems, with over 3,000 known species worldwide^{1–3}. Although much is known about how parasitic plants influence host performance^{1–4}, their role as drivers of community- and ecosystem-level properties remains largely unexplored⁵. Parasitic plants have the potential to influence directly the productivity and structure of plant communities because they cause harm to particular host plants, indirectly increasing the competitive status of non-host species^{6–10}. Such parasite-driven above-ground effects might also have important indirect consequences through altering the quantity and quality of resources that enter soil, thereby affecting the activity of decomposer organisms^{3,11–13}. Here we show in model grassland communities that the parasitic plant *Rhinanthus minor*, which occurs widely throughout Europe and North America¹⁴, has strong direct effects on above-ground community properties, increasing plant diversity and reducing productivity. We also show that these direct effects of *R. minor* on the plant community have marked indirect effects on below-ground properties, ultimately increasing rates of nitrogen cycling. Our study provides evidence that parasitic plants act as a major driver of both above-ground and below-ground properties of grassland ecosystems.

Rhinanthus minor is a facultative root hemiparasite that is commonly associated with low to medium fertility grasslands¹⁵ (see Supplementary Fig. 1) and is known to infect fast-growing grasses as a preferred host, thereby reducing their competitive dominance¹⁶. As a consequence, the use of *R. minor* has been proposed as a management tool for restoring botanical diversity to agriculturally improved grassland^{8,17}. We set up mesocosms of mixed grassland communities on soils of varying residual fertility, based primarily on soil phosphorus (P) availability, ranging from low, to low–medium, to medium fertility (see Methods and Supplementary Table 1). Grassland communities were constructed by sowing into field soil a number of grass (*Agrostis capillaris*, *Alopecurus pratensis*, *Briza media*, *Lolium perenne*, *Phleum bertolonii*, *Poa trivialis*) and forb (*Geranium sylvaticum*, *Lotus corniculatus*, *Ranunculus acris*, *Ranunculus bulbosus*, *Ranunculus repens*) species typical of traditionally managed grassland¹⁸. To these, three *R. minor* treatments were applied to represent a range of natural field densities (that is, none, medium density at 30 plants m⁻², and high density at 60 plants m⁻²)^{8,14}, along with three manure treatments (that is, none, 12 tonnes ha⁻¹ alternate years, and 12 tonnes ha⁻¹ yr⁻¹). This factorial design of treatments allowed us to test, in soils of varying residual fertility, the influence of *R. minor* on community properties relative to normal farming practice of applying manures¹⁹.

Total above-ground biomass (excluding *R. minor*) measured three years after treatments had started was reduced significantly by high

densities of *R. minor* ($F_{2,54} = 4.72$, $P = 0.013$) (Fig. 1a). Furthermore, there were no significant interactions with residual soil fertility or manure application (Table 1), indicating that the suppressive effect of *R. minor* on above-ground biomass occurred irrespective of

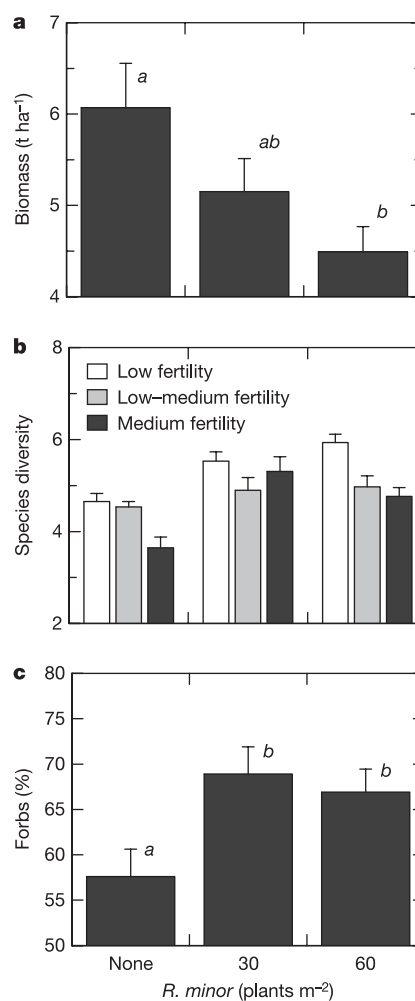


Figure 1 | Mean (\pm s.e.m.) values for plant community parameters as affected by the presence of *R. minor* after three years. **a**, Biomass of above-ground plant material, excluding *R. minor*. **b**, Plant diversity, excluding *R. minor*, calculated using the inverse Simpson's index. **c**, Proportion of forbs, excluding *R. minor*, within the plant community. Values with different letters are significantly different at the $P < 0.05$ level.

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Table 1 | Above-ground community properties after three years of experimental treatments

Source	Species composition		Above-ground biomass		Diversity	
	Per cent*	F (P-value)	Per cent*	F (P-value)	Per cent*	F (P-value)
Main treatment effects						
FYM addition	3.1	1.74 (NS)	1.3	0.62 (NS)	5.0	3.73 (0.031)
<i>R. minor</i> addition	19.2	10.89 (0.002)	9.9	4.72 (0.013)	26.0	19.52 (<0.001)
Residual fertility	11.5	6.51 (0.002)	4.0	1.94 (NS)	14.6	10.89 (<0.001)
Two-way interactions						
<i>R. minor</i> × residual fertility	6.0	1.76 (0.012)	1.1	0.25 (NS)	8.2	3.06 (0.024)
<i>R. minor</i> × FYM addition	3.0	0.85 (NS)	7.6	1.82 (NS)	6.1	2.29 (NS)
FYM × residual fertility	2.3	0.62 (NS)	0.8	0.19 (NS)	0.7	0.27 (NS)
Three-way interaction						
FYM × <i>R. minor</i> × soil residual fertility	5.3	-	18.8	2.25 (0.038)	3.4	0.63 (NS)
Residual and block variance	49.6	-	56.5	-	36.0	-

* Per cent variation attributable to treatment.
FYM, farmyard manure; NS, $P > 0.05$.

variation in these factors. Although above-ground biomass was greatest ($F_{2,54} = 47.78$, $P < 0.001$) on the most fertile soils and in pots receiving manure ($F_{2,54} = 19.33$, $P < 0.001$) after one year, these responses were no longer detected after three years (Supplementary Table 2). Despite this, shoot tissue P concentration (a proxy for soil P availability) for the grass *L. perenne* and forbs *G. sylvaticum* and *R. acris* was lowest in the low fertility soil (Supplementary Table 3). Also, root colonization of these three plant species with arbuscular mycorrhizal fungi, which is typically highest when P is deficient²⁰, was consistently greater in the low fertility soil (Supplementary Table 3). Together, these findings indicate that initial differences in soil fertility, in terms of P availability, continued throughout the experiment.

Associated with a decline in above-ground productivity was a significant increase in plant diversity ($F_{2,54} = 19.52$, $P < 0.001$) in the presence of *R. minor* (Fig. 1b). Plant species diversity was also affected significantly by residual soil fertility, being greatest in the low fertility soil ($F_{2,54} = 10.89$, $P < 0.001$) (Fig. 1b). There was also a significant interaction ($F_{4,54} = 3.06$, $P = 0.024$) between the effects on plant diversity of residual soil fertility and *R. minor*: plant diversity was always lowest at zero *R. minor* for any given soil fertility, and was always largest in the low residual fertility soil at any given *R. minor* level, but the significance of differences between these treatments were not consistent (Fig. 1b). The application of manure resulted in a significant increase in plant diversity ($F_{2,54} = 3.73$, $P = 0.031$). Analysis of individual plant responses to *R. minor* revealed that its positive effect on plant diversity was due to a marked suppression of the competitively dominant grass *L. perenne* ($F_{2,54} = 41.3$, $P < 0.001$), and an increase in the abundance of the forbs *G. sylvaticum* ($F_{2,54} = 17.61$, $P < 0.001$) and *R. acris* ($F_{2,54} = 35.76$, $P < 0.001$) (Table 2). As a consequence, *R. minor* markedly increased ($F_{2,54} = 4.57$, $P = 0.015$) the proportion of forbs within the community (Fig. 1c). This is consistent with the prediction that *R. minor* will increase grassland diversity through suppression of competitively dominant grasses, which this species preferentially infects¹⁶. The abundance of these grass and forb species was unaffected by the application of manure, but this treatment did significantly decrease the proportion of forbs within the community ($F_{2,54} = 3.91$, $P = 0.026$).

The significance of *R. minor* as a driver of plant community composition, relative to the other factors of residual soil fertility and manure application, is highlighted by the fact that most variance in measures of plant community biomass and composition was attributable to its presence; only a small proportion of variance was attributed to residual fertility and manure, and interactions between experimental factors (Table 1). These data suggest, therefore, that within low-to-moderately productive mesotrophic grasslands, where *R. minor* typically occurs¹⁵, parasite-driven effects can be equal to, or greater than, those of other factors such as residual soil fertility and manure application.

Producer and decomposer subsystems function in tandem to maintain ecosystem functioning^{21–23}. Therefore, we expected above-ground responses to *R. minor* to be associated with changes in decomposer processes and organisms. To test this, we measured soil microbial community responses to *R. minor* and other treatments using phospholipid fatty acid analysis (PLFA) (see Methods). Total PLFA was unaffected by experimental treatments, indicating that the size of the microbial community was unresponsive. However, we found that the structure of the microbial community changed. In particular, the ratio of fungal-to-bacterial fatty acids was lowest in the most fertile soil ($F_{2,54} = 8.98$, $P < 0.001$), which is consistent with the notion that fungi are relatively more abundant and functionally important than bacteria under less fertile conditions^{22,23} (Fig. 2a). The fungal-to-bacterial biomass ratio measure was also reduced by the presence of *R. minor*, albeit with marginal significance ($F_{2,54} = 2.53$, $P = 0.09$) (Fig. 2b), suggesting that changes in the plant community resulting from *R. minor* infection set a trend in motion towards increasing bacterial dominance in the microbial community.

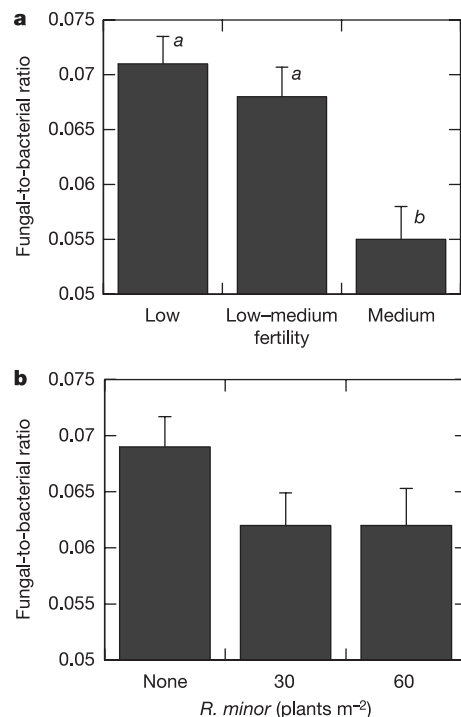


Figure 2 | Mean (\pm s.e.m.) values for fungal-to-bacterial biomass ratio, calculated using PLFA, after three years. a, Influence of soil residual fertility. b, Influence of *R. minor*. Values with different letters are significantly different at the $P < 0.05$ level.

Table 2 | Significant treatment effects of *R. minor* on the biomass of two forbs and a grass after three years

Species	<i>Rhinanthus minor</i> population		
	None	30 plants m ⁻²	60 plants m ⁻²
<i>Geranium sylvaticum</i>	77.0 ^a	313.8 ^b	286.6 ^b
<i>Ranunculus acris</i>	327.5 ^a	896.2 ^b	870.6 ^b
<i>Lolium perenne</i>	1,872.0 ^a	664.3 ^b	592.3 ^b
Other species	3,793.5	3,555.7	2,970.4

Values are in kg ha⁻¹, and different letters indicate that values are significantly different at the $P < 0.05$ level. Other species refers to the total biomass of all other species that showed no response to the *R. minor* treatment plus the biomass of *R. minor*. *G. sylvaticum* and *R. acris* are forbs; *L. perenne* is a grass.

Increases in the abundance of bacteria relative to fungi in the soil microbial community are typically associated with an increase in rates of nutrient cycling^{22–23}. In accordance with this, we detected a significant enhancement in soil nutrient cycling due to parasite presence. In particular, we found that the rate of nitrogen (N) mineralization increased markedly ($F_{2,54} = 11.72$, $P < 0.0001$) in the presence of *R. minor* (Fig. 3a); N mineralization increased by 105% and 174% at low and high hemiparasite densities, respectively. As a consequence, the availability of mineral N (DIN) relative to dissolved organic N (DON) also increased, being almost twice as high in the presence of *R. minor* (Fig. 3b). This was due to a significant increase in soil concentrations of mineral N in soil solution, the preferred N source for most plants of temperate grassland communities²⁴. Surprisingly, rates of N mineralization and soil concentrations of DIN did not vary significantly with residual soil fertility, but the concentration of DON in soil solution was found to be lowest ($F_{2,54} = 6.24$, $P < 0.004$) when residual soil fertility was low, perhaps indicating lower N availability in this treatment (data not shown). The lack of difference in DIN in soils of varying residual fertility, however, probably reflects the depletion of available pools of soil inorganic N due to plant growth over the course of the experiment.

The magnitude of the parasite-driven increase in N mineralization

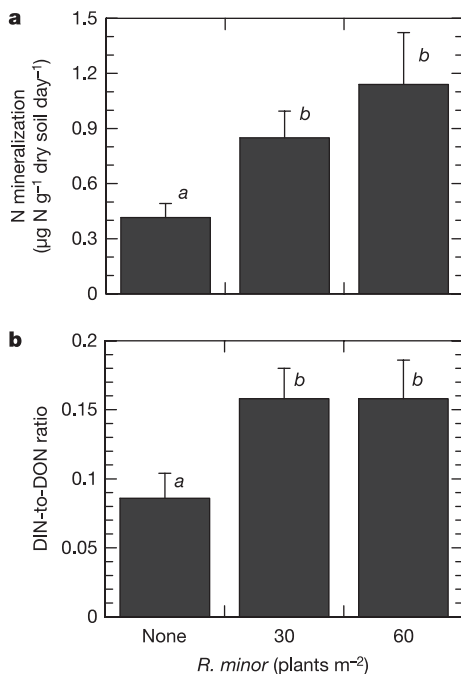


Figure 3 | Mean (± s.e.m.) values for soil N cycling parameters as affected by the presence of *R. minor* after three years. a, Rates of N mineralization. b, Ratio of DIN to DON. Values with different letters are significantly different at the $P < 0.05$ level.

shown here might be expected to feed back to the plant community in terms of increased above-ground productivity and reduced diversity, due to promotion of competitive, fast-growing grasses at the expense of herbs²⁵. That this was not detected points further to the powerful suppressive effect of *R. minor* on competitive, fast-growing grasses and its dominant role as a driver of plant diversity in grassland. In the longer term, however, we expect that indirect effects of parasitic plants on below-ground properties will have far reaching consequences for plant nutrition and production in grassland. The enhancement of soil N cycling in the presence of *R. minor* is most likely an indirect consequence of changes in the quality and quantity of plant inputs to soil, resulting from shifts in the productivity and composition of the sward. It is likely that parasite-driven changes in root growth and turnover, and root exudation patterns, will have altered carbon supply to soil, thereby affecting the activity of decomposer organisms and nutrient mineralization. It has been suggested that parasitic plants might positively influence soil nutrient cycling through their ability to accumulate nutrients in their leaves, thus producing high quality litter that decomposes rapidly, releasing nutrients that would otherwise remain in the host plant or in slowly decomposing litter^{11–13}. This is unlikely to be the case here because the N content of *R. minor* was low compared to other species (see Supplementary Table 4), and the presence of *R. minor* in the sward had no effect on the N content of other species. Also, most above-ground tissue was harvested, so the return of senescent litter to the soil was minimal.

Our findings illustrate that parasitic plants act as major drivers of both the structure and function of grassland ecosystems. They also bolster growing evidence of the importance of above-ground consumers of primary productivity as indirect modulators of ecosystem properties through their influence on below-ground organisms and their activities^{21–23,26}, and specifically illustrate the importance of parasites for understanding ecosystem dynamics⁵. The mechanisms involved in parasite-driven enhancement of nutrient cycling are poorly understood. However, given that almost all terrestrial ecosystems support parasitic plants^{1–4,14}, they are likely to have substantial, but largely unexplored, effects in many ecosystems.

METHODS

Mesocosm design. The mesocosm experiment was set out in March 2000 at the University of Newcastle field station, Close House, England (latitude 54° 59' N, longitude 1° 48' W, elevation 30 m). Mesocosms were 42-l polypropylene pots with a 10-cm base of carboniferous limestone chippings. Each was filled with one of three soils to provide the residual fertility treatments that were a consequence of different past fertilizer practices on the same clayey alluvial soil (Fladbury series) at three sites in Dentdale, Yorkshire (see Supplementary Table 1). Low fertility soil, previously receiving an annual application of farmyard manure, was collected from a traditionally managed hay meadow at Scotchergill Farm (latitude 54° 16' N, longitude 2° 26' W, elevation 138 m). Low-medium fertility soil, previously receiving the same farmyard manure application plus about 25 kg ha⁻¹ of NPK mineral fertilizer, was collected from a hay meadow at Northwaite Farm (latitude 54° 16' N, longitude 2° 25' W, elevation 150 m). A moderate fertility soil, previously receiving slurry and 50 kg ha⁻¹ of NPK mineral fertilizer, was also collected from Northwaite Farm, from a field cut twice a year for silage (latitude 54° 16' N, longitude 2° 24' W, elevation 160 m).

The initial flush of seedlings from the seed bank was removed in May 2000. Mesocosms were then sown with *A. pratensis*, *L. perenne*, *L. bertolonii*, *P. trivialis* and *R. repens*, species characteristic of productive grassland. In September 2000 they were sown with *A. capillaris*, *B. media*, *G. sylvaticum*, *L. corniculatus*, *R. bulbosus* and *R. acris*, species characteristic of traditionally managed northern meadows¹⁸. The *R. minor* treatments (none, 30 and 60 plants m⁻²) were applied in September each year by sowing seeds into appropriate mesocosms. Three farmyard manure treatments (none, 12 tonnes ha⁻¹ alternate years and 12 tonnes ha⁻¹ every year) were based on UK government management prescription for traditional meadows within UK Environmentally Sensitive Areas¹⁹. Composted farmyard manure (total nutrient content: 27.8 mg N g⁻¹; 15.4 mg P g⁻¹; 36.1 mg K g⁻¹) was applied in January of each year. Each treatment combination was replicated three times to give 81 mesocosms, laid out in three blocks. Each of the three treatment replicates was randomly positioned in each block. Artificial autumn grazing was applied each year by randomly

trampling the soil surface of each mesocosm with an artificial 'hoof'. Throughout spring and early summer in every year, all unsown species were removed from the mesocosms, and *R. minor* was thinned out to the required treatment density. **Plant and soil measurements.** The sward was cut annually in July and total dry weight of harvested vegetation was recorded. Dry weight of each species was recorded in July 2003. The vegetation within the central 25 × 25 cm grid was cut with shears and hand-sorted to species, expressed as kg ha⁻¹ dry weight. Air-dried plant material of individual species was used for multi-element analysis by a single Kjeldahl micro-digestion²⁴. At the final harvest, roots of selected plant species were taken for determination of root colonization by arbuscular mycorrhizal fungi²⁷. In July 2003, five soil samples were taken from each mesocosm, bulked, passed through a 4-mm sieve, and stored at 4°C until analysis. Soil microbial community structure of these samples was assessed using phospholipid fatty acid analysis²⁸, and soil N availability was measured using three techniques²⁴. First, concentrations of mineral N (DIN) in soil solution were determined by auto-analysis. Second, net N mineralization was measured as the release of mineral N (NH₄⁺-N and NO₃⁻-N) after incubation of samples for 14 days at 25°C. After incubation, soil concentrations of mineral N were determined. Third, dissolved organic N (DON) was measured by oxidation of water extracts with potassium persulphate (K₂S₂O₈), and measurement of the resultant mineral N by autoanalyser procedures.

Data analysis. Main treatment effects on biomass of the most frequent plant species, and on the total biomass each year, were assessed by analysis of variance (ANOVA) using MINITAB. Interactions between treatments were tested as part of the ANOVA. Where a significant difference was found with the ANOVA test, the significance of differences between means was tested using Tukey's test. When a species was absent from one treatment, analysis was based on a reduced number of treatments. Analysis of variance was used to assess treatment differences for soil biological variables. This was also done for the N, P and K concentration in the above-ground parts of the main plant species. A test of normality, the Anderson–Darling test, was applied to the residuals of each analysis. If this deviated significantly from normality then a suitable transformation was applied to the original data. The proportion of the variability in 2003 species composition attributable to each treatment, and its significance, were based on ordination methods using CANOCO²⁹. The same data for yield and diversity were based on the sums of squares associated with each treatment in an ANOVA test.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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