Journal of Biogeography (*J. Biogeogr.*) (2004) **31**, 415–424

ORIGINAL ARTICLE

Soil characteristics of Rocky Mountain National Park grasslands invaded by *Melilotus officinalis* and *M. alba*

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ABSTRACT

Aim Invasion of nitrogen-fixing non-native plant species may alter soil resources and impact native plant communities. Altered soils may be the driving mechanism that provides a suitable environment to facilitate future invasions and decrease native biodiversity. We hypothesized that *Melilotus* invasion would increase nitrogen availability and produce soil microclimate and biochemical changes, which could in turn alter plant species composition in a montane grassland community.

Location Our research addressed the effects of white and yellow sweet clover (*Melilotus officinalis* and *M. alba*) invasion on soil characteristics and nitrogen processes in the montane grasslands in Rocky Mountain National Park.

Methods We sampled soil in replicate sites of *Melilotus*-invaded and control (non-invaded) patches within disturbed areas in montane grassland habitats. Soil composites were analysed for available nitrogen, net nitrogen mineralization, moisture, carbon/nitrogen (C : N ratio), texture, organic matter and pH. Data were recorded at three sample dates during the growing seasons of 1998 and 1999.

Results Contrary to our expectations, we observed lower nitrogen availability and mineralization in invaded patches, and differences in soil moisture content and soil C : N. Soil C : N ratios were higher in invaded plots, in spite of the fact that *Melilotus* had the lowest C : N ratios of other plant tissue analysed in this study.

Main conclusions These findings provide land managers of natural areas with a better perspective on the possibilities of nitrogen-fixing species impact on soil nutrient levels.

Keywords

Biological invasion, soil biogeochemistry, soil nitrogen, *Melilotus officinalis*, *Melilotus alba*.

INTRODUCTION

Non-native invasion and nitrogen fixation are mechanisms that can alter soil conditions and nutrient levels. For instance, leguminous nitrogen-fixing plants may alter soil nitrogen availability and thus change some soil properties (Chapin *et al.*, 1986; Vitousek *et al.*, 1987). Researchers have found nitrogen-fixing species to affect soil and species communities in a variety of ecosystems (Chapin *et al.*, 1986; Vitousek & Walker, 1989; Maron & Connors, 1996; Simons & Seastedt, 1997; Thomas & Bowman, 1998). Watt (1947) described how the regeneration of species in grasslands could facilitate other species establishment. In addition, changes in soil characteristics may inhibit vegetation spread (Beatty, 1989). This can lead to community processes that are altered and resources fragmented within the landscape (McGill & Cole, 1981; Vitousek & Walker, 1989; Asner & Beatty, 1996). Current research demonstrates the importance of nitrogen-fixing non-native plants on ecosystem properties and function. For instance, some nitrogen-fixing plants can create negative effects on plant communities in nutrient-poor ecosystems. In Hawaii, one introduced nitrogen-fixing tree, *Myrica faya,* rapidly invades forests and outcompetes *Metrosideros polymorpha,* a native tree, for light and nutrients (Vitousek &

Walker, 1989). In South Africa, a non-native nitrogen-fixing *Acacia* tree outcompetes native trees for water, light and other nutrients (Witkowski, 1991). In turn, size of nutrient and energy pools in soil and vegetation can be permanently altered (Vitousek, 1990; Christian & Wilson, 1999).

Human activities, such as roadsides or construction, can redistribute soil nutrient availability and other soil properties such as soil texture and moisture. This type of land use change can encourage exotic invasion by shifting the storage and flux capacities of carbon and nitrogen (Vitousek *et al.*, 1997). One example of this is in agricultural lands, which typically have low C : N ratios in most crops. Low C : N ratios prevent long-term C storage per unit of N input. In some cases, nutrient-rich soils facilitate certain non-native invaders (Stohlgren *et al.*, 1999). However, in nutrient-poor soils that have high C : N ratios, non-native species that are nitrogen fixers easily exploit the area and spatially outcompete native species (Vitousek & Howarth, 1991). Soil resources can ultimately be altered by nitrogen-fixing plants that use microbe-filled root nodules to transform atmospheric and organic nitrogen to a usable form of inorganic nitrogen (Davidson *et al.*, 1979; Vance, 1991; Brady & Weil, 1996). Because they have higher nutrient use efficiency (NUE), native species can persist in these soils, but nitrogen-fixing invaders often maintain lower NUE. NUE is a measure of how a plant responds to soil nutrient levels, defined as the amount of organic matter lost or produced in a plant per amount of nutrient used (Vitousek, 1982). Depending on whether plants have high or low NUE, they can influence nutrient return to the soil and litter quality (Schlesinger, 1997). In the montane zone, most cool-season native grasses have high NUE and slower growth because of a lower capacity to capture resources (Chapin *et al.*, 1986; Chapin, 1991; Morgan, 1994). Many non-native species exhibit low NUE which allows them to grow quickly and exploit resources. Higher NUE in non-native plants can lead to altered, in some cases lowered, nutrient availability for native species. Soil biota can also play a role in nutrient availability. For example, earthworms can increase nutrient availability which can facilitate non-native plant into forests and reduce native plant abundance (Hendrix & Bohlen, 2002). Within the rhizosphere, soil fungi can be an important pathway for nutrient uptake for non-native plants (Callaway *et al.*, 2003).

Because nitrogen-fixing opportunistic invaders can thrive in nutrient-poor disturbed soils, their invasion is often fostered by anthropogenic disturbances. Although nutrient availability can facilitate invasion, abiotic soil factors can also play a role as a driving mechanism for invasion. In roadside and construction soils, the texture is often sandier than the surrounding soils. As sand content increases, soil moisture and nutrient availability decreases. Sandier soil is subject to rapid nutrient loss depending on weathering, and nutrients are limited compared with clayey soils (Tilman, 1987; Vitousek & Walker, 1989). As a result, species that can persist in lower nutrient soils establish in these areas.

Soil disturbance creates opportunities for exotic invaders but it is unclear if establishment is due to altered nutrient availability or if the invader affected nutrient availability (Elton, 1958). When resources become altered or limited, species dynamics shift depending on a plant's response to limiting resources (Tilman, 1987). For example, nitrogen-fixing invaders that compete with native species for resources can grow quickly or invest in nitrogen-fixing microbes, allowing them to exploit or alter resource availability. Thus, changes in limiting resources can lead to a shift in dominant species (Seastedt & Knapp, 1993; Simons & Seastedt, 1997).

This research examined nutrient and abiotic soil properties in areas that were invaded by the non-native species, *Melilotus officinalis* and *M. alba*. Because they are highly adaptable to a wide range of soil conditions, *Melilotus* species can grow well in nutrient-poor and calcareous soils as well as in drought conditions (Duke, 1981). These species have a symbiotic relationship with *Rhizobium* bacteria which permits *Melilotus* species to grow in nitrogen-depleted soils. As opportunistic biennials, *Melilotus* species possess characteristics that make them competitive in nature and alter soil characteristics. They (1) colonize disturbed areas, (2) produce a large number of seeds, (3) grow early in the season to create a tall dense colony, (4) produce extensive root systems and (5) fix nitrogen for their immediate use.

Legumes can be rare in montane grasslands, unless they are planted (Vitousek, 1990; Ritchie & Tilman, 1995). As a result, *Melilotus* species are highly invasive on the Northern Great Plains (Lesica & DeLuca, 2000). Although it originated in Eurasia as a foraging plant, it is found useful in other countries, including Australia and South America. Because of its deep tap roots and low water use efficiency, *Melilotus* has been found to restore salt-affected roadsides. In Argentina, the plant can restore moderately saline soils to crop-worthy soils. In some studies, these species are associated with understorey plant assemblages in cottonwood and ash communities (Girard *et al.*, 1989a,b). However, these species may have the potential to alter native species composition as in North America (Royer & Dickinson, 1999; Wolf *et al.*, 2003). In the US and Canada, it is considered a pandemic in certain regions, but is not a problem in other regions (Smoliak *et al.*, 1981). However, seeds can be imported from other countries to North America, and the problem is perpetuated.

Typically, *Melilotus* species are found in disturbed soils created by human activity, where the most common land use is agriculture (Duke, 1981). Disturbed soil provides a rough surface for germination, a critical requirement of scarification for *Melilotus* seeds. In an agricultural setting, *Melilotus* species are perceived as having a positive impact on soil. These species are intentionally planted with non-nitrogen-fixing species to enhance soil nitrogen. However, in a natural ecosystem, high nitrogen availability may not benefit native species (Maron & Connors, 1996).

In a study in a Wisconsin prairie, Parker *et al.* (1993) found *M. alba* growing in disturbed soil that had higher pH, lower organic matter and lower nitrogen. When these species dominate native plant communities, *Melilotus* can ultimately outshade the native species (Cole, 1991). However, *Melilotus* species do not persist well in shaded sites. It may be implied then, that in certain forest conditions, native species can outshade *Melilotus* species. But if a plant community becomes dominated by nitrogen fixers, it can change nutrient availability and ecosystem function through biogeochemical processes (Melillo & Gosz, 1983; Simons & Seastedt, 1997).

Invasibility can depend on many factors, such as the level of space and nutrient availability (Stohlgren *et al.*, 1999). In this study, soils within patches of *Melilotus*- and non-invaded (control) montane grassland were compared to assess the potential of *Melilotus* species to act as spatial and resource competitors in Rocky Mountain National Park (RMNP) and to determine whether *Melilotus* species can impact available soil resources. First, we hypothesize that available soil nitrogen and net mineralization would be higher in invaded patches compared with control patches. Secondly, we hypothesize that moisture would be lower in invaded patches between patches because *Melilotus* grow earlier and quicker than the native species and would exploit moisture resources faster than native species. Thirdly, we hypothesize that soil pH and C : N would be lower in invaded patches as a result of *Melilotus* contribution to the soil rhizosphere.

Study site

This research was conducted in the montane zone (2316–2743 m) on the east side of RMNP in Colorado, USA (study area map in Wolf *et al.*, 2003). Because of the relatively moderate climate, human impact and soil disturbance is high in these montane grasslands. In various places in RMNP, soil was disturbed several years ago when it was used as a homestead and guest ranch (Buchholtz, 1983). The site locations were dictated by the occurrence of *M. officinalis* in May which were found along human disturbances such as roadsides, filled ditches and building construction. In this study, the sites were located in the montane zone, the lowest elevation in RMNP. For each *Melilotus*-invaded patch, one edge terminated into a road or trail and was subjected to full sun conditions.

The RMNP montane life zone (2316–2743 m) has an average annual precipitation of 54 cm, with most precipitation in July and August. The soils in this zone support grasslands and meadows, open stands of ponderosa pine (*Pinus ponderosa*) and juniper (*Juniperus scopulorum*) on south-facing slopes, Douglas fir (*Pseudotsuga menziesii*) on north-facing slopes, riparian areas and aspen (*Populus tremuloides*) groves. In this life zone, burrowing animals also create natural soil disturbances, and mule deer and elk may distribute soil nitrogen in certain areas when their population numbers increase during the summer, as grazing can stimulate mineralization (Hogan, 1993; Frank & Groffman, 2003).

In the study sites, the soils do not appear to have a profile, and in some instances may be mixed with fill dirt from outside the Park. However, a complete soil survey is currently being conducted by the NRCS, but is not yet available. At a coarse scale, Park soils are Cryochrepts, Cryoboralfs and Cryaquepts. The Cryochrept is a well-drained soil with moderately rapid permeability and slow run-off, found in the glacial till areas. These soils are deep to very deep, with some large stones and boulders on the surface and in the profile. The Cryoboralfs are moderately-well to well-drained with moderate permeability and run-off. The erosion hazard is slight. The Cryaquepts are poorly drained, with slow to moderate permeability and run-off. They are found in wetter, flatter areas. The erosion hazard is slight unless slopes are denuded of vegetation. At the finer scale of this study's profile, study sites under the native community examined in this research are Alfisols; however, the disturbances leave the epipedon more sandy, dry and nutrient-poor, like an Aridisol. Soils are composed of schists and granite. The soils appear to be shallow, with rock appearing at 30–40 cm depth in some cases. Thus, the soils may be highly influenced and created by erosion debris from glacial till and more recently, river alluvium.

Montane grasslands occur on well-drained soils and they are co-dominated by native grasses and forbs (Peet, 1978). Of the over 1000 documented plants in RMNP, 120 are non-native. Common native grasses and forbs specific to this location include: *Muhlenbergia montana*, *Bouteloua gracilis*, *Stipa comata*, *Koeleria macrantha*, *Poa agassizensis*, *Pseudoroegneria spicata*, *Artemisia frigida*, *Eriogonum umbellatum*, *Penstamon virgatus*, *Grindelia subalpina*, *Heterotheca vollosa*, *Helianthus pumilus*, *Chrysothamnus vicidoflorus* and *Potentilla hippiana* (Wolf *et al.*, 2003).

METHODS

To set up the sampling design, we located 16 sites that were already invaded by *Melilotus* in May. To control for the least amount of variability, we choose 12 sites because the invaded and control patches were equally impacted by the same disturbance type, duration, and intensity, sunlight level, aspect and slope. Both within and beyond the patch boundaries, the plant community appeared equivalent except for the presence of *Melilotus.* Because this study investigates the effect of nitrogen fixers, it is important to note that two native nitrogen-fixing plants, *Thermopsis divaricarpa* and *Astragalus flexuoses* were found in only two sites, but when they were present, they occurred in both invaded and control patches (Wolf *et al.*, 2003). We choose sites that were located near Park Headquarters, the research building and employee residential area, in Moraine Park, Alluvial Fan and Aspenglen. The size of each patch ranged from 25 to 75 m^2. During the growing seasons of 1998 and 1999, two soil composites (10 soil cores each) were extracted with soil augers from random locations within a grid that covered the entire invaded and control patch every 6 weeks from May to September. All soil composites were analysed for available inorganic nitrogen (NH_4^+ and NO_3^-), net nitrogen mineralization, pH, organic matter, colour, moisture, temperature and texture (at 0–10 and 10–20-cm depths). We determined the soil colour classification of one composite from each site (Munsell Color Company, Inc., 1954). Soil temperature differences could result from *Melilotus* cover in the invaded patches.

We determined available inorganic nitrogen content (NH_4^+ and NO_3^-) by treating a subset of the field moist soil composites with a 1 : 3 mixture of soil to KCl (1 N). The extracts were then shaken and filtered. Colorimetric methods were used to determine available NH_4^+ concentrations in the extractant (with a precision of ± 0.2%). A subsample from each extractant was used to determine available NO_3^- (Lachet Instruments 1989). We analysed net nitrogen mineralization as an index of nitrogen availability and microbial activity. The mineralization process involved the following steps: a portion of each soil composite was placed in a sealed bag, then buried at 10 cm for 4 weeks in the same collection location. At the end of the incubation period, each bag was retrieved and analysed in the same way as the original composites. In addition, the net mineralization calculations were corrected for gravimetric water content.

Because net mineralization and immobilization by microbial activity are often associated with the soil organic carbon to nitrogen ratio (C : N) (McGill & Cole, 1981; Wedin & Tilman, 1990), we estimated C : N ratio from soil composites and foliage specimens taken from each patch from dry combustion on a LECO-1000 CHN analyzer (Leco Corporation, St. Joseph, MI, USA). In this procedure, soil samples and leaves from *Melilotus* plants, two native grasses and two native forbs were ignited in the analyzer. In addition, ICP spectrometry analysis was used to measure other elemental concentrations, such as phosphorus. Soil acidity (pH) was measured in a 1 : 1 soil/water solution, while organic matter was determined by loss on ignition (680 °C).

To determine soil moisture, 30 g of soil were weighed out from each soil composite and oven-dried for 8 h in a convection oven (105 °C) to determine gravimetric percentage soil moisture by weight. Soil temperature was recorded to observe correlation with soil moisture. Temperature readings were measured with a soil temperature probe at soil depths of 5, 10 and 15 cm in invaded and control patches. The presence or absence of rain or snow was recorded.

We analysed soil texture using the Boyoucous hydrometer method (ASTM standard test method D42263). International Society for Soil Science values were used to determine the soil separates. In this process, we used a muffle furnace to ash the soil subsamples (580 °C) to remove organic matter and residual moisture. The ashed material was then sifted through a 2-mm sieve to remove gravel-sized particles.

Data analysis

Because our data were taken after the *Melilotus* invasion occurred in the patches, this study represents a space-for-time experimental design. We compared the following physical and biological soil properties between invaded and control patches using analysis of variance (ANOVA). Repeated measures ANOVA were tested for differences in means of available ammonium and nitrate (NH_4^+ and NO_3^-), net mineralization for ammonium and nitrate, pH, organic matter, moisture, *in situ* soil temperature at three depths, on three sample dates (May, July and August) (SAS Institute, 1989). Although some variables may be naturally intercorrelated, we relied on ANOVA to extract trends through time and differences between patches. Two variables, texture and C : N ratio, were tested on their mean values with a one-way ANOVA at one time period. In the repeated measures analysis, a polynomial contrast was used to assess the linear and quadratic changes over time. This technique accounts for temporal autocorrelation that may exist in data recorded at three time periods (Johnson, 1998). Autocorrelation plays an important role in ecological patterns, and it may be that nested plots within random plots are more powerful than obtaining complete random sampling in a heterogeneous area (Stohlgren *et al.*, 1995). The nested ANOVA is an effective analysis for the fixed treatment effect within random sites. It partitions the variance, where SS for treatment and interaction (the mixed model ANOVA case) is divided into SS as a result of treatment within each level of the random factor. A one-way ANOVA then obtains statistics for individual treatments, while a two-way ANOVA obtains these statistics for the random effect. Separate *t*-tests may give the same results as a nested ANOVA, but this is often undesirable because pooled standard error relies on less data for each test (less degrees of freedom), and site differences cannot be evaluated (McKone & Lively 1993).

RESULTS

Available nitrogen

In both growing seasons of 1998 and 1999, we found significantly less available ammonium and available nitrate (NH_4^+ and NO_3^-) in the invaded patches compared with the control patches ($P < 0.002$ for NH_4^+ and $P < 0.09$ for NO_3^-) (Fig. 1). During these years, NH_4^+ levels remained low in invaded patches throughout the season, but the levels fluctuated in the control patches. Available NO_3^- was also lower in invaded patches ($P > 0.02$). In May, the beginning of the growing season, NO_3^- was higher in all patches but progressively decreased by August (Fig. 1). However, compared with the invaded patches, NO_3^- was significantly higher in the control patches, and remained higher from July in both years to August in 1998. Nitrate averaged 0.12 mEq 100 g^{-1} soil in invaded patches and 0.16–0.17 mEq 100 g^{-1} soil in control patches in 1999.

The repeated measures reveal that net mineralization of NH_4^+ in control patches was higher compared with invaded patches during June and July ($P < 0.0001$), but there was no difference in August of 1998 (Fig. 2). In 1999, soil bags for the May incubation period were found destroyed in the field, so these bags were deleted from the analysis; however, we still had enough intact bags to analyse. Net nitrification in 1998 was higher in June and September in the control patches; however, higher levels occurred in invaded patches in July 1998 ($P < 0.06$). Net nitrification in 1999 was higher in invaded plots in June, but in July and September, nitrification was higher in control plots.

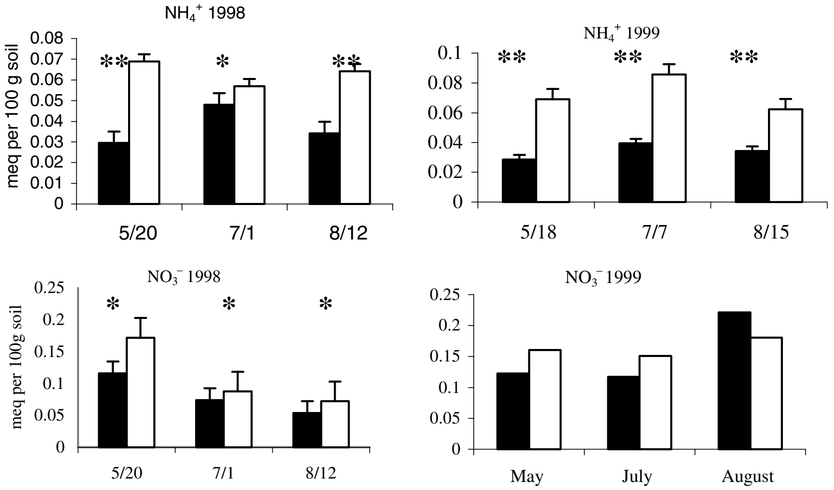

Figure 1 Mean concentrations (mean + 1 SE) of available ammonium ($n = 71$) and available nitrate ($n = 72$) (mEq 100 g^{-1} soil) in *Melilotus*-invaded and control patches in growing season in montane grasslands in Rocky Mountain National Park in 1998 and 1999. ■, invaded patches; □, control patches. In all cases but 15 August 1999, higher available nitrogen occurred in control patches. Repeated measures analysis showed a significant difference ($P < 0.02$) for NH_4 in both years. Between patches show significantly lower available nitrogen for all tests except for nitrate in 1999, $^*P < 0.02$, $^{**}P < 0.01$.

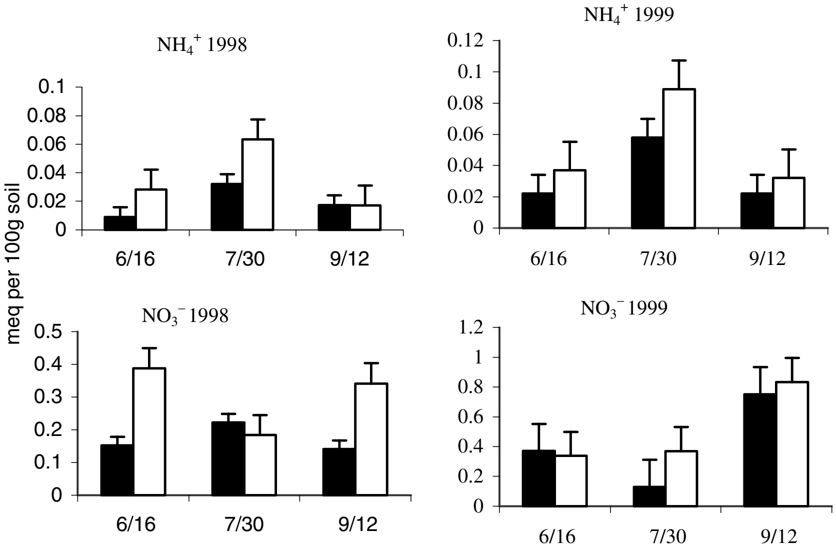

Figure 2 Mean concentrations (mean + 1 SE) of net mineralization of ammonium ($n = 59$) and nitrate ($n = 70$) (mEq 100 g^{-1} soil) in *Melilotus*-invaded and control patches throughout growing season in 1998 and 1999. ■, invaded patches; □, control patches. Between patches, higher net mineralization activity was observed in control patches, but differences were not significant ($P > 0.01$). NH_4 mineralization increased in July in both years.

Soil carbon nitrogen ratio

The soil C : N ratio did not differ between control and invaded patches in 1998 ($P < 0.1556$), but the ratio was higher in invaded patches in 1999 ($P < 0.011$) (Fig. 3). The C : N in grass ($P < 0.399$) and forb species ($P < 0.334$) did not differ between patches. In 1999, C : N in *M. officinalis* tissue was less than half that of grasses or other forbs.

Soil organic matter and soil pH

Differences in soil organic matter depended on the depth. Overall, soil organic matter ranged from 1.60% to 10.74% of mass in invaded patches, and from 3.80% to 12.57% of mass in the control patches. At 10–20 cm depth, the mean percentage of soil organic matter was significantly lower (3.96, $n = 47$) in invaded patches [$F(1,41) = 4.23$, $P < 0.046$], compared with control patches (6.22). However, at 0–10 cm, organic matter was the same between patches (5.12 invaded, 6.96 control) ($P < 0.356$).

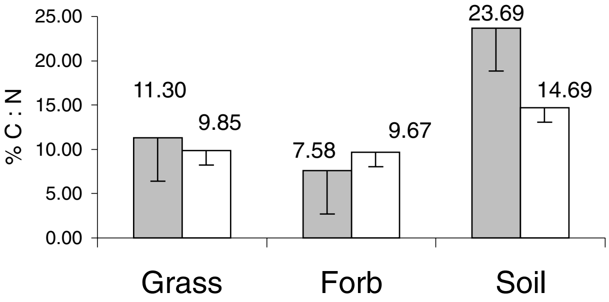

Figure 3 Total carbon to nitrogen ratio in foliage (grass and forbs) and soil (0–10-cm depth) in invaded and control patches in 1998. ▨, invaded patches; □, control patches, $n = 14$. Soil C : N was slightly higher in invaded patches compared with the control patches, $P < 0.15$. Grass = 0.399, forbs = 0.334, soil = 0.155. Error bars represent −1 pooled SE of the mean estimate. In 1999, *Melilotus* C : N = 9.33, grass (17.22 invaded/17.26 control), forbs (17.05 invaded/14.22 control = 0.63), $P < 0.509$, soil (19.08 invaded/13.16 control), $F = 15.49$, $P < 0.011$.

Table 1 Mean soil pH in invaded and control patches throughout the growing season

	Invaded	Control	*F*(dfn, dfd)	*P*<
1998 (*n* = 26)				
20 May	6.91	6.46	0.95(1,11)	0.38
1 July	6.77	6.29	3.27(1,11)	0.145
12 August	7.00	6.41	3.96(1,11)	0.121
1999 (*n* = 20)				
20 May	7.07	6.674	0.04(1,26)	0.94
6 July	6.295	5.49	4.16(1,16)	0.06
14 August	6.602	6.564	1.97(1,16)	0.18

The repeated measures analysis showed that soil pH was significantly lower in control patches in July 1999 (Table 1). In addition, while pH did not differ significantly at other times, soil pH in invaded sites was always higher than in the control sites, and a decrease in pH occurred in July for both years.

Soil moisture, temperature and texture

Soil moisture was lower in invaded patches compared with control patches in May and August 1998, and soil moisture decreased at 5- and 10-cm depths ($P < 0.001$). In all patches, soil moisture dramatically decreased in the annual dry interval in June/early July and patches had equal moisture. We found higher soil moisture in invaded patches in May, late July and August, and low values in both patches on 1 July 1998 (Fig. 4). In 1998, soil moisture was higher in August compared with July, but in 1999, soil moisture was lower in August compared with May. Soil temperature steadily decreased with depth. Temperature did not differ between patches, although it was slightly higher in invaded patches compared with control patches.

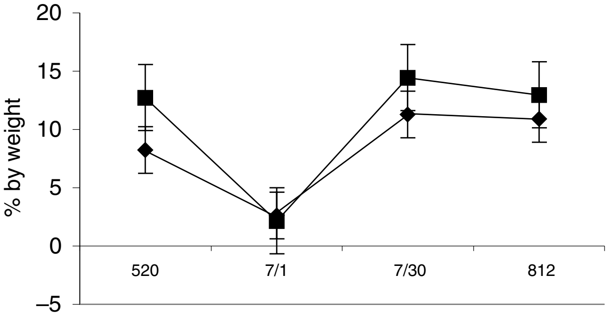

Figure 4 Mean gravimetric soil moisture in *Melilotus*-invaded and control patches throughout the growing season in 1998 (in figure) and in 1999 (text below). ◆, invaded patches; ■, control patches. Six-week recording: 20 May–1 July; 4 weeks, 1–30 July; 2 weeks, 30 July–12 August. Error bars represent ±1 pooled SE of the mean estimate. Repeated measures in 1998 = [$F(1,15) = 146.74$, $P < 0.0001$]. Note convergence as soil moisture decreases in both patches during the drought period on 1 July ($P < 0.089$). At this time, moisture was not different between patches; however, moisture was higher at all other times in control patches ($P < 0.0001$). On 20 May 1999, invaded patch = 10.36, control = 7.91; on 6 July 1999, invaded = 1.43, control = 1.36; on 14 August 1999, invaded = 5.11, control = 8.37.

Table 2 Percentage sand, silt and clay by weight in *Melilotus*-invaded and control patches at two depths

	0–10 cm		10–20 cm		
	Invaded	Control	Invaded	Control	*P*<
Sand	86.97	83.08	87.37	84.79	0.069
Silt	9.94	13.15	9.25	11.47	0.057
Clay	3.17	3.80	3.37	3.75	0.056

$n = 47$, $F = 2.21(1,19)$, standard error is −1 pooled SE of the mean estimate. *F*-tests are not independent, because texture is a three-component closed system (sand, silt and clay must always equal 100%).

Soil textures were sandy and sandy-loams (Table 2). The silt content was slightly, but non-significantly higher ($P > 0.06$), at both depths (mean of 87% in invaded vs. 83.4% in the control patches) in invaded patches. Clay content did not differ between patches (3.9% in control patches vs. 3.2% in invaded patches) ($P < 0.056$).

DISCUSSION

Biotic factors: nutrients, C : N, pH, organic matter and roots

Although our first hypothesis was not confirmed, it was reasonable to hypothesize that higher nitrogen levels would occur in invaded patches. Surprisingly, we found lower available nitrogen in invaded patches. These findings were surprising as *Melilotus* species is a nitrogen-fixing plant (Turkington *et al.*, 1978; Duke, 1981). We confirmed the presence of root nodules on our specimens, and we have no reason to believe that the nitrogen-fixing mechanism was not active during the period of this study.

There are several reasons to believe that *Melilotus* played a role in soil nitrogen differences between the invaded and non-invaded patches. First, all the patches were similar with regard to texture, colour and disturbance history. Secondly, the *Melilotus* has been in these particular places within the Park for at least 14 years (J. Connor, Park Research, pers. comm.). Thirdly, as *Melilotus* thrives within a wide range of soil texture, moisture, elevational and climatic regime, it is not expected that a lower nitrogen level would facilitate *Melilotus* distribution patterns found in this study. Disturbance may be a more important factor; this notion is particularly important to park managers because of the risk that *Melilotus* may expand into non-invaded areas (Wolf *et al.*, 2003). Thus, the lower nitrogen availability found in the invaded patches could result from the plant's aggressive growth pattern, long growing season and low NUE. The ability to provide its own nitrogen may allow this species to drive the soil nutrient levels down due to quick consumption and growth. As a consequence, once

Melilotus species invade disturbed environments, they may impede colonization or survival of native species, and facilitate their own persistence. The lower NO_3^- levels in invaded patches is expected due to both the lower levels of NH_4^+ and reduced nitrification. But higher net mineralization in control patches can also respond to the relatively higher level of nitrogen found in these patches (Hobbs & Schimel, 1984; Vitousek & Walker, 1989). In high nutrient use efficient species, a competitive strategy in nutrient-poor conditions is to retranslocate stored nitrogen from the leaves during senescence or at the end of the growing season (Chapin *et al.*, 1986; Chapin, 1991; Parker *et al.*, 1993).

Lower available nitrogen and net mineralization in invaded patches is associated with the higher soil C : N found in invaded patches in 1999. Higher C : N in invaded patches may be due to higher amounts of carbon inputs in root detritus or a reduction of demposition in root detritus. Lower C : N in *Melilotus* plant tissue compared with both grass and forb plant tissue that were collected in the same time period suggests that *Melilotus* may use nitrogen more quickly than native species in the montane grasslands. However, it should be noted that the biennial and seasonal growth patterns of *Melilotus* may create fluctuations in nutrient availability.

Because *Melilotus* species grow well on calcareous soil, it is not surprising that soil pH is near neutral. In most cases, invaded patches were more alkaline than control patches. However, the near-neutral pH may lead to decreased phosphorus availability. We found significant differences in phosphorus between invaded and control patches (4.944 mg kg^{-1} in invaded vs. 9.954 in control patches). Levels of nitrogen and phosphorus in soil can be closely tied (Evans & Belnap, 1999), and one could expect lower available phosphorus in these soils to act as a possible inhibitor of invasion, because phosphorus availability often limits nitrogen fixation (Brady & Weil, 1996). Higher available phosphorus in control patches may suggest a way to inhibit *Melilotus* invasion.

Root size and structure can change nutrient availability (Mooney *et al.*, 1987) which can influence species composition. In this study, most of the roots that were excavated and analysed for nodules had the longest lateral roots in the upper two-thirds of the taproot, which can be important in nodulation processes (Wolf & Rohrs, 2001). Finally, soil resource heterogeneity is strongly influenced by vegetation (Partel & Wilson, 2002). For instance, the degree of fine roots, root length and root turnover can influence root competition and lead to higher spatial variability and heterogeneity in soil resources.

Abiotic factors: soil moisture, temperature and texture

Our results confirmed the second hypothesis regarding differences in soil moisture between invaded and control patches. Lower moisture in invaded patches could be due to *Melilotus* quick growth pattern and longer persistence leading to faster evapotranspiration. One might hypothesize that the plant's presence created a higher percentage ground cover, so evaporation would be expected to be lower under the canopy of the plants in the invaded patches, resulting in higher soil moisture. In May, *Melilotus* species already had a higher percentage ground cover than any other early season species. Percentage cover of *Melilotus* persisted throughout the growing season, and into October, in the invaded patches. However, other non-native species that are fast growing and provide a high level of ground cover have been associated with lower soil moisture (Brown & Archer, 1999).

In addition to high *Melilotus* productivity, moisture loss in invaded patches could also be a function of soil texture (Webb *et al.*, 1978; McLendon & Redente, 1991). As soils become sandier, their ability to hold moisture at shallow depths decreases. One may argue that the slightly higher sand content in the invaded patches and higher clay content in the control patches could explain why there is less moisture in *Melilotus*-invaded patches. Nutrients are affected by soil texture; soils with higher clay content have higher nutrient availability. As invaded patch soils had a higher sand content, low nutrient levels would be expected. However, the difference in sand and clay content between patches was at most marginally significant ($P = 0.06$). Although soil texture could be treated as a partial cause to explain the difference in soil moisture and nutrient levels; the C : N ratio and other life-history characteristics of *Melilotus* may indicate that these species play a role in altered soil resources within invaded patches.

Broader implications of *Melilotus* invasion are the association of these species with disturbance, and the spread of these species into native communities. *Melilotus* are associated with disturbed soils (Turkington *et al.*, 1978); once established, individual *Melilotus* plants may dominate the plant community (Wolf *et al.*, 2003). And because *Melilotus* can exist on a wide range of soil textures, it may be able to expand into the native community if enough bare soil is present to scarify the seeds. In time, root growth during the first year (Cole, 1991) can assist *Melilotus* to expand into undisturbed native communities.

Although this study is an observational comparison that utilizes a space-for-time design, which can limit the ability to draw causal relationships, the research offers insight to managers to whether soil effects may be due to or responsible for *Melilotus* invasions, and are valuable for generating hypotheses for future experimentation. Important implications are that: (1) lower soil nitrogen was associated with *Melilotus* presence, (2) these species may alter soil moisture levels and (3) these species may spread beyond disturbed soils. This has implications for persistence of native species composition. In a vegetation analysis, invaded patches were different in species composition compared with control patches. More non-native species were found in invaded patches, although species richness was the same between patches (Wolf *et al.*, 2003). Non-native grass and forb species found in both invaded and control patches were *Bromopsis inermis*, *Phleum pratense*, *Bromus tectorum*, *Agropyron smithii*, *Agropyron cristatum*, *Lepidium virginium y virginicum*, *Arabis hirsute*, *Alyssum minus* and *Achilla millefolium*; however, certain non-native species, such as *Erigeron flagellaris*, *Sisymbrium altissimum*, *Rumex crispus*, *Tragopogon porrifolius*, *Agrostis stolonifera* and *Verbascum thapsus* were found only in invaded patches. Additionally, certain native species found in control patches, such as *Selaginella densa*, *Astragalus flexuoses*, *Phacelia hastate*, *Gaillardia aristata*, *Heterotheca villosa*, *Oligosporus pacificus*, *Stipa comata*, *Stipa viridula* and *Geranium richardsonii* were never in invaded patches. Park managers may use these data to better understand the role of soil and consequent plant community changes by non-native invasion.

Melilotus species are known to be associated with other weedy species (Turkington *et al.*, 1978), so it is important to investigate whether *Melilotus* species facilitate the soil for other species, or if other species prepare the soil to allow *Melilotus* colonization. Similarly, it is important to determine if other species that invade nutrient-poor areas can deplete nitrogen further. Because they can persist at lower soil nitrogen levels, nitrogen-fixing species play an important role in succession (Robertson & Vitousek, 1981; Ritchie & Tilman, 1995; Tilman *et al.*, 1996). Finally, testing techniques that may eliminate or control *Melilotus* and minimize soil disturbance would benefit National Park managers. Preserving the rigour of native communities through the processes of control techniques is a critical focus for Park restoration efforts. In identifying invasive non-native species that may impact soil conditions, Park managers can better control their populations, especially if they are still in low numbers.

Our study illustrates the importance of focusing attention on soil characteristics in the presence of nitrogen-fixing non-native invasive species. This is especially important for montane soils in National Parks for several reasons. First, the dry climate and thin, immature, sandy soils support native plant assemblages that can exist at low nitrogen levels. In developing nodules to fix nitrogen, *Melilotus* may be expected to alter the nitrogen distribution in the ecosystem which could ultimately shift the community composition and spatial structure in the nitrogen-rich patches. Our results are unique in that we were able to quantify a variation from a predicted increased nitrogen. Secondly, our study is also of interest because it reveals the potential for non-native species to significantly alter soil resources. Finally, this research benefits Park managers who strive to minimize soil disturbance and invasion spread in a context of changing land use to accommodate the growing numbers of visitors. This action is important if non-native species continue to invade roadsides and construction sites and eventually encroach the roadless habitats (Gelbard & Harrison, 2003). As more roads and buildings are created, the landscape could reflect plant community changes from invasion that began in the disturbed zones.

ACKNOWLEDGMENTS

This research was supported by the National Science Foundation, Rocky Mountain Nature Association and the National Park Service. Other funding came from the Edna Bailey Sussman Fellowship, the Colorado Mountain Club and the University of Colorado Museum. We thank Jeff Connor for help in initiating this research in the Park. For comments and intense editing on the manuscript, we thank Margie Krest. For research assistance, we thank Jennifer Rohrs, Christine Edwards, Carl Cordova, Dan Campbell, Jeff Lukas, Emily Friend, Connor Bailey, Brenton Wonders and Jeremy Goff. We thank Chris Seibold at the Mountain Research Station for NO_3^- analysis assistance and Jim Sells at Colorado State University for extended conversations and assistance with soil techniques.

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