Nitrogen Uptake during Snowmelt by the Snow Buttercup, *Ranunculus adoneus*

**R. B. Mullen,**  
**S. K. Schmidt,**  
**and**  
**C. H. Jaeger III**

Department of Environmental,  
Population and Organismic Biology,  
Campus Box 334, University of  
Colorado, Boulder, Colorado 80309,  
U.S.A. schmidts@spot.colorado.edu

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**Abstract**

Seasonal patterns of nitrogen (N) uptake were measured to assess the ability of the alpine herb *Ranunculus adoneus* to utilize the flush of N during snowmelt in an alpine tundra ecosystem. Development of mycorrhizal and dark septate fungi were also monitored within the roots of this snowbed plant in order to determine the role of these fungi in N acquisition. In addition, soil temperature, moisture, and inorganic N levels were measured to determine possible influences of edaphic factors on plant N uptake. In contrast to P uptake, which occurs late in the growing season and corresponds with arbuscular mycorrhizal (AM) development, N uptake occurred very early in the growing season before new roots and active AM fungal structures were formed. Soils at this time were cold and wet and NH$_4^+$ was the predominant form of inorganic nitrogen. Our data indicate that *R. adoneus* is able to take advantage of the early season flush of N by utilizing the previous year’s root system which is heavily infected with a dark septate fungus. Given the high density of *R. adoneus* plants (135 plants per m$^2$) in this snowbed system, they are a significant sink (ca. 0.5 g N per m$^2$) for nitrogen released from within or beneath the alpine snowpack.

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**Introduction**

*Ranunculus adoneus* (the snow buttercup) emerges in full bloom through snow at the edges of long-lived snowbanks at elevations of approximately 3000 to 4000 m in the Rocky Mountains. The environment of these snowbanks is characterized by low soil temperatures and a short growing season (Billings and Bliss, 1959) and *R. adoneus* possesses a suite of attributes which allow it to complete its life cycle in this environment (Stanton and Galen, 1989; Mullen and Schmidt, 1993; Scherff et al., 1994). For instance, flowers of *R. adoneus* are heliotropic, thus receiving the most benefit of sunlight and therefore insect pollinators throughout the day (Stanton and Galen, 1989). In addition this plant accumulates phosphorus (P) after seed set when soil temperatures are relatively high. This P is then stored over the winter and re-mobilized early in the following season allowing *R. adoneus* to begin growing while soil temperatures are close to 0°C and the plants are still covered with snow (Mullen and Schmidt, 1993). Phosphorus uptake coincides with the development of arbuscular mycorrhizal (AM) fungi in the roots of *R. adoneus* indicating that these fungi are important to the P nutrition of wild populations of *R. adoneus* (Mullen and Schmidt, 1993).

Nitrogen (N) can be an important limiting nutrient to plant growth in tundra systems (Chapin, 1980; Giblin et al., 1991; Kielland and Chapin 1992; Bowman et al., 1993; Jonasson et al., 1993). Nitrogen levels in the soil beneath alpine snowpacks are highest just prior to, and during, snowmelt (Brooks et al., 1996). This flush of N is probably in large part due to over winter release of N from organic matter and microorganisms (Kielland, 1990; DeLuca et al., 1992; Chapin et al., 1993; Brooks et al., 1996). However, the fate of this N in alpine systems has not been determined. Studies in more temperate systems indicate that early spring N can be immobilized by soil microorganisms and to a lesser extent by plants (Zak et al., 1990). In addition, it has recently been demonstrated on Niwot Ridge, Colorado that the some of early season N pulse is immobilized by soil microorganisms (Brooks et al., 1996, 1997). Before the present study, the role of plants in immobilizing this early season pulse has not been examined in any high elevation tundra ecosystem.

Our goal was to determine to what extent *R. adoneus* could accumulate N released during snowmelt. We hypothesized that N uptake would not be dependent on new root growth or AM fungi because these structures develop after the initial flush of N (Mullen and Schmidt, 1993). We followed the temporal patterns of N uptake, root development and fungal colonization of new and old roots of *R. adoneus*. This was done in a wild population of *R. adoneus* while we simultaneously measured edaphic factors over an entire growing season. In addition to providing information about the physiological ecology of *R. adoneus* and its fungal partners, this study also provides valuable information about the fate of the early season N pulse that occurs in many alpine systems.

**Methods**

**STUDY SITE**

The site for this study was Niwot Ridge, a Long-Term Ecological Research Site (LTER) in the Colorado Front Range (40°03’N, 105°36’W). The saddle on Niwot Ridge contains an east-facing snowbed (3510 m elevation) which remains partially unmelted until mid-August. *Ranunculus adoneus* is common throughout this snowbed and is one of the few plants that is active during snowmelt (Mullen and Schmidt, 1993). The sampling sites are on a slope (ca. 20°) and snowmelt water runs through the site for most of July. Snow usually starts to accumulate at this site in October. The snowbed soil was characterized as a typic cryumbrept with 6 to 16% organic matter and a pH of 4.5 to 5.0 (Burns 1980).
PLANT AND SOIL ANALYSES

During June and July, when plants were growing most rapidly, plants were collected biweekly. Toward the end of the growing season plants were collected monthly. Collections consisted of six plants which were chosen as randomly as possible, but also with the objective of selecting plants of approximately the same size and therefore, age. Each plant was removed with sufficient soil to ensure that the entire root system was obtained. Once collected, plants and soil were transported immediately to the laboratory and were stored at 4°C for a maximum of 24 h before processing. Processing consisted of washing and separating plants into new roots, old roots, and shoots. New roots were placed in preservative (as below) and old roots and shoots were dried, weighed, ground and analyzed for total Kjeldahl nitrogen using a hydrogen peroxide and salicylic acid digest (Haynes, 1980) and colorimetric analysis by a flow-injection analyzer (FIA) (LACHAT Instruments, Mequon, Wisconsin, USA).

Soil temperature was measured at each sampling date at depths of 5 and 10 cm using a Li-Cor datalogger with thermocouple probes. Soil moisture was measured gravimetrically by drying to constant weight at 60°C to determine water content. In addition, soil NH₄⁺ and NO₃⁻ were determined by extraction of fresh soil in a 2M KCl solution followed by analysis using a Lachat FIA.

Density of R. adoneus at the study sites was quantified using 20 × 20 cm squares that were tossed randomly (N = 62) within the study area. The mean number of plants per quadrant was used to calculate the density of plants per square meter.

Time-course data were analyzed using ANOVAs. Bonferroni multiple comparisons were performed to test for significant differences among data (Judd and McClelland, 1989).

QUANTIFICATION OF MYCORRHIZAL DEVELOPMENT

New roots from each plant were measured for length, and stored in F.A.A. (90% formalin, 5% acetic acid, 5% ethanol). On three sampling dates old roots from previous years were collected and also placed in F.A.A. Roots were stained as described in Mullen and Schmidt (1993). Roots were cleared in 10% KOH in a boiling water bath for at least 1 h, acidified in 1% HCl for 20 min, and stained in cold lactophenol trypan blue (0.05%) for at least 12 h. Quantification of fungal infection was done in a manner similar to that of McGonigle et al. (1990). Roots were cut into 2-cm sections and arranged lengthwise on microscope slides. Passes were made vertically across the slide at regular intervals using a compound microscope (160×). When roots were crossed an ocular crosshair was aligned perpendicular to the long axis of the root and the presence or absence of fungi was recorded for the area directly under the crosshair. If the crosshair intersected fungal structures, the type of fungus and fungal structures were recorded. The magnification used was primarily 160×, although identification of specific structures sometimes required using a magnification of 400×. Using this approach it was possible to quantify the stages of mycorrhizal development in addition to total infection levels for each type of fungus in the root. Infection is expressed as a percent of total root length infected for each type of fungal structure.

Results

Preliminary measurements from 1990 indicated that N uptake occurred very early in the growth cycle of R. adoneus, prior to our earliest sampling dates (data not shown). Thus, during the 1991 season we began sampling when flowers of R. adoneus first appeared through the melting snowpack in June. Seasonal patterns of N accumulation and depletion in the shoots and roots of R. adoneus during 1991 are presented in Figure 1. During the early part of the growing season a net uptake of N into plant tissues was observed in both roots and shoots of R. adoneus (Fig. 1). Some N also appears to have been translocated from roots to shoots. Percent N in the shoots of R. adoneus increased significantly (P < 0.05) from late June to early July and this rise coincided with a drop in percent N in the roots (Fig. 1b). Even accounting for this translocation of N from roots to shoot a large increase in N uptake was observed from 25 June to 8 July (Fig. 1c). This early season uptake of N for the most part preceded net shoot growth by R. adoneus. Shoots continued to increase in mass until late July (Fig. 2) whereas net N accumulation had ceased by 8 July (Fig. 1).

Edaphic factors may have influenced N uptake by R. adoneus. N uptake occurred when soil temperature was rising and soil moisture was declining (Fig. 3). In addition, NH₄⁺ was the dominant inorganic N species in the soil during the early season (Fig. 3c).

Because of the unique rooting phenology of R. adoneus (Mullen and Schmidt, 1993), we were able to follow the patterns of new root and mycorrhizal development in the same plants from which the shoot N data were taken (Fig. 4). New root growth reached its peak during mid-July (Fig. 4a) at which time arbuscular mycorrhizal (AM) infection was just beginning to increase (Fig. 4b). Thus, N accumulation occurred before significant new root growth and well before AM fungi developed in these new roots (Figs. 1b, 1c).

Arbuscular mycorrhizal and other fungal infection levels also were monitored in old roots (i.e. roots that had over-wintered) of R. adoneus plants collected on three of the same dates.
that the N measurements were made. Extremely low levels of arbuscules were found in these old roots at all sampling dates, but a dark septate (DS) fungus (Haselwandter and Read, 1982; Trappe, 1988; Väre et al., 1992; Mullen and Schmidt, 1993) was present at high levels early and late in the growing season (Fig. 5).

Discussion

Our interest in *R. adoneus* stemmed from our curiosity about nutrient acquisition by plants as alpine snowbeds melt. *Ranunculus adoneus* begins growth prior to other snowbed plants, thus we hypothesized that N uptake by *R. adoneus* would occur at the beginning of its growing season during the early season N flush observed by Brooks et al. (1996) at this site. In addition, we also hypothesized that N uptake, in contrast to P uptake (Mullen and Schmidt 1993) would not be dependent on new root growth or AM fungi because these structures develop well after the initial flush of N. These hypotheses were substantiated in the present study.

Our results show that *R. adoneus* is able to take advantage of the seasonal pulse of N availability. Recent studies at our site indicate that N availability is very high just prior to and during snowmelt (Brooks et al., 1996). Our results show that N accumulation occurred very early in the life cycle of *R. adoneus*, tripling the amount of total N in plant tissues within 2 wk of emergence. This net accumulation of N occurred when soil temperatures ranged between 0 and 8°C (Fig. 3a) and when soils were still wet from snowmelt (Fig. 3b). During this time snowmelt from the upper part of the snowbed was still flowing through our site. In comparison, other alpine plants accumulate N somewhat later in the growing season although they also tend to accumulate more N in the first half of the growing season than in the second half (Jaeger et al., 1997). Of the plants studied to date on Niwot Ridge, however, *R. adoneus* seems to be alone in its ability to exploit the very early flush of N associated with snowmelt.

Our data indicate that early season N uptake by *R. adoneus* may significantly impact N losses from this alpine system. Our estimate that *R. adoneus* can immobilize approximately 0.53 g N m⁻² is similar to the level of N removal by a spring ephemeral community in a deciduous forest of 0.55 g m⁻² (5.5 kg ha⁻¹) (Blank et al., 1980) and higher than the early season immobilization of N (0.1 g m⁻²) noted by Muller and Bormann (1976) for *Erythronium americanum* at Hubbard Brook.

In order to investigate the mechanism of early season N uptake by *R. adoneus*, we quantified the developmental patterns of roots and mycorrhizal fungi associated with this plant. A number of studies have examined the presence of mycorrhizae in the genus *Ranunculus* (Mullen and Schmidt, 1993; Read and Haselwandter, 1981; Väre et al., 1992, 1997), but none of these studies examined the functional role of these fungi in the nitrogen nutrition of *Ranunculus* spp. In addition, there have been very few previous studies relating the development of root systems to functioning of alpine plants (Barnola and Montilla, 1997; Mullen and Schmidt, 1993). In the present study, maximal N accumulation in the field occurred at a time when almost no new roots were observed on *R. adoneus* plants. Thus, we concluded that new roots were not involved in early season N uptake by *R. adoneus*. It is also possible to rule out a significant role for AM fungi in the early season N nutrition of *R. adoneus*. The peak in arbuscule levels in new roots of *R. adoneus* occurred well after the period of maximal N accumulation. Although the old roots of *R. adoneus* contained AM hyphae (data not shown), arbuscules were very scarce in these roots early in the season (Fig. 5). Because arbuscules are the site of nutrient transfer between the fungus and plant (Smith and Smith 1989), a lack of arbuscules is a strong indication that no nutrient transfer was taking place between AM fungi and *R. adoneus* early in the growing season.

In contrast to arbuscules, there were high levels of a dark septate (DS) fungus in the over-wintered roots of *R. adoneus* early in the season. Dark septate fungi are very common in high elevation plants, but very little work has been done to determine the functional role of these fungi and their role in N nutrition of wild plants remains unknown (Haselwandter and Read, 1982; Stoyke and Currah, 1991; Wilcox and Wang, 1987). Haselwand-
ter and Read (1982) showed that DS fungi can significantly increase P uptake and growth of the alpine sedge, Carex firma, but they did not test the effects of the DS fungus on N nutrition of that plant. In another alpine sedge, C. sempervirens, however, Haselwandter and Read (1982) showed no effect of the fungus on plant growth and speculated that a nutrient other than P may have been limiting the growth of this plant in the P-deficient sand system that they used.

The extent to which DS fungi may take up N or impart nutrients to host plants is not known. At our site, N and P are usually the limiting nutrients for plant growth (Bowman et al., 1993). Thus, at a time when soils are relatively cold, new roots are not yet functional, old roots are highly suberized (indicating a decrease in nutrient uptake efficiency), and active AM structures are absent, R. adoneus is taking up large amounts of nitrogen from surrounding soils. One explanation for this uptake is that the DS fungus contributes to uptake of inorganic and organic N by this plant. Mycorrhizal fungi have been shown to aid in uptake of organic N (Abuzinadah and Read, 1986; Bajwa and Read, 1986) but this has not been demonstrated for DS fungi. We have recently isolated the DS fungus from R. adoneus and have shown that it can take up and utilize both NH₄⁺ and organic forms of N at temperatures as low as 2°C (Mullen and Schmidt, unpublished data). Thus, the DS fungus has the potential to act as a conduit for both organic and inorganic forms of N at temperatures experienced by early season roots of R. adoneus. They also produce sclerotia, fungal storage structures, within the cortical cells of R. adoneus roots, which could be digested by the plant cells. The role of these fungi deserves more investigation given that these they are extremely common in plant roots and soil and rarely cause pathogenic effects.

In conclusion, R. adoneus takes up the majority of its N early in the season when snowmelt is occurring at and just uphill from the plants. These results may help explain why very little N is lost from vegetated sites in the alpine even early in the growing season (Brooks et al., 1996). The role that fungal endophytes play in this early season retention of N needs more study.

Acknowledgments

We thank D. Lipson, T. Raab, and A. Yen for field and technical assistance and Chris Schadt for comments on the manuscript. This research was supported by EPA grant R82-3442-01, NSF grant IBN95-14123 and grants from Sigma Xi and the Spokane Mushroom Club.

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Ms submitted August 1997