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4   Temporal Variation in Nutrient Uptake Capacity by Intact Roots of Mature

5   Loblolly Pine

6

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2   loblolly pine, nutrient uptake, nutrient uptake capacity, seasonality

3

4   **Abstract**

5   Nutrient uptake is generally thought to exhibit a simple seasonal pattern, but few

6   studies have measured temporal variation of nutrient uptake capacity in mature

7   trees. We measured net uptake capacity of K, NH_4^+, NO_3^-, Mg and Ca across a

8   range of solution concentrations by roots of mature loblolly pine at Calhoun

9   Experimental Forest in October 2001, July 2001, and April 2002. Uptake

10   capacity was generally lowest in July; rates in October were similar to those in

11   April. Across a range of concentrations, antecedent nutrient solution

12   concentrations affected the temporal patterns in uptake in July but not in October

13   or April. In July, uptake of NH_4^+, Mg and Ca was positively correlated with

14   concentration when roots were exposed to successively lower concentrations,

15   but negatively correlated with concentration when exposed to successively

16   higher concentrations. In contrast, uptake in October was constant across the

17   range of concentrations, while uptake increased with concentration in April. As in

18   studies of other species, we found greater uptake of NH_4^+ than NO_3^-. Temporal

19   patterns of uptake capacity are difficult to predict, and our results indicate that

20   experimental conditions, such as experiment duration, antecedent root conditions

21   and nutrient solution concentration, affect measured rates of nutrient uptake.

22

1 **Introduction**

2 The seasonality of nutrient uptake by roots affects fertilizer use efficiency, soil

3 solution concentrations, and stream export of nutrients. For example, the timing

4 of fertilizer application to fast-growing trees, such as loblolly pine (*Pinus taeda*

5 L.), is critical to maximize nutrient uptake and storage capacity (Miller, 1981;

6 Johnson and Todd, 1988). Since some studies have shown that less than 15%

7 of nitrogen fertilizer applied may be retained by trees (Heilman and Gessel, 1963;

8 Van Miegroet *et al.*, 1994), understanding what controls the timing of uptake is

9 important to minimize nutrient leaching into groundwater and streams and to

10 maximize fertilizer use efficiency.

11   Plant growth and foliar nutrients vary during the growing season (Nelson

12 *et al.*, 1970; Adams *et al.*, 1987; Valentine and Allen, 1990), but these patterns

13 may not reflect seasonal patterns of nutrient uptake because a significant fraction

14 of nutrients are remobilized from within the plant (Millard and Proe, 1992; Proe *et*

15 *al.*, 2000). Such remobilization enables plants to grow during periods of low

16 nutrient availability and uptake (Millard, 1994).

17   Temporal patterns of ion uptake in trees have been measured primarily

18 using seedlings, and simple seasonal trends have been observed. Nitrogen

19 uptake of young *Pinus radiata* (D. Don) in the field was higher in spring and

20 summer than autumn and winter (Smethurst and Nambiar, 1989). Phosphorus

21 uptake of three-year-old *Picea sitchensis* (Bongard) Carrière grown in the

22 greenhouse was similar in spring and late summer (Proe and Millard, 1995).

1   Mature trees may differ from seedlings in their temporal pattern of uptake,

2   since plant age affects root anatomy (Eissenstat and Achor, 1999; Wells and

3   Eissenstat, 2003), root respiration (Wells and Eissenstat, 2003), and nitrogen

4   and phosphorus uptake capacity (Dong *et al.*, 2001; Wells and Eissenstat, 2003).

5   Only one study to date has examined temporal trends in uptake using intact roots

6   of mature trees. Net ammonium uptake was highest in the summer in subalpine

7   *Fagus sylvatica* L. (beech) and *Picea abies* (L.) Karst (spruce) (Gessler *et al.*,

8   1998).

9   Temporal patterns in nutrient uptake through the year are driven by

10   variations in soil nutrient availability and the plant's capacity to take up nutrients.

11   These factors are not independent, since plants regulate uptake capacity based

12   on nutrient supply (Lee, 1982; Drew *et al.*, 1984; BassiriRad *et al.*, 1993). Plants

13   have high NO_3^- uptake rates following a period of deficiency (Lee and Rudge,

14   1986; Siddiqi *et al.*, 1989), and exhibit low uptake after exposure to high NO_3^-

15   concentrations, due to saturation of exchange sites at the root surface (Dean-

16   Drummond, 1982; Siddiqi *et al.*, 1990).

17   We examined nutrient uptake capacity in a commercially important conifer,

18   loblolly pine, by exposing roots to known concentrations of nutrient solutions .

19   Using this approach, changes in uptake capacity over time can be measured

20   independent of variation in ambient soil solution concentrations and soil moisture

21   conditions. The objective of our study was to quantify temporal variation in

22   potassium, ammonium, nitrate, magnesium and calcium uptake capacity by

23   measuring net uptake across a range of nutrient concentrations in July, October

1	and April. We hypothesized that uptake capacity would decline between late

2	summer and fall and then increase to a maximum in spring. We also examined

3	how uptake differed with experimental conditions, such as nutrient solution

4	concentration, experiment length and antecedent nutrient solution concentration.

5	We expected net nutrient uptake rates to increase with nutrient solution

6	concentration and experiment duration. We also predicted that uptake capacity

7	would be higher when plants were given low antecedent nutrient solution

8	concentrations. Finally, we compared the time course of NH_4^+ and NO_3^- uptake,

9	predicting that uptake of NH_4^+ would be more rapid than that of NO_3^-.

10

11	**Materials and methods**

12	*Study area*

13	This study was conducted in the Calhoun Experimental Forest of the Sumter

14	National Forest in Union County, South Carolina (82 $^\circ$N, 34.5 $^\circ$W). Annual

15	precipitation averages 1228 mm (1971-2000) and mean annual temperature is

16	15 $^\circ$C (Administration, 2002). Soils are well-developed Ultisols of the series

17	Appling and Cataula (clayey, mixed, thermic Typic Kanhapludults, (Overstreet

18	and Bell, 1965). Soils are acidic with low exchangeable Ca and Mg, especially in

19	the upper 15 cm (0.05 cmol$_c$ kg^{-1} Ca and 0.02 cmol$_c$ kg^{-1} Mg) (Markewitz *et al.*,

20	1998). Average concentrations of NH_4-N and NO_3-N were low in soil solutions

21	collected from the forest floor (35 µmol$_c$ L^{-1} NH_4-N and 10 µmol$_c$ L^{-1} NO_3-N) and

22	from 15 cm (8 µmol$_c$ L^{-1} NH_4-N and 3 µmol$_c$ L^{-1} NO_3-N). Virtually no nitrogen in

23	either form was detected below 60 cm depth (Markewitz *et al.*, 1998).

1 Cumulative precipitation was 285 mm, 350mm, and 463mm for the two weeks

2 preceding our measurements in July, 2001, October 2001, and April 2002,

3 respectively (NOAA Administration, 2002). Mean daily high (low) temperatures

4 were 28ºC (19 ºC) in July; 19 ºC (7 ºC) in October and 19 ºC (5 ºC) in April.

5   Cotton (*Gossypium hirsutum* L.), corn (*Zea maize* L.) and wheat (*Triticum*

6 *aestivum* L.) were grown at the site for many decades prior to 1954 (Urrego,

7 1993). Loblolly pine seedlings were planted at Calhoun in 1956. The rate of

8 biomass accumulation was highest from 1966-1976, and total biomass reached a

9 peak of 211-247 Mg ha^{-1} in 1984 (Markewitz *et al.*, 1998). Total live biomass has

10 recently declined, reaching 174 Mg ha^{-1} in 2000 (D. Richter, pers. comm.) as a

11 result of mortality from southern pine beetle (*Dendroctonus frontalis*) attacks and

12 wind damage from heavy storms.

13

14 *Overview of methods*

15 Nutrient uptake capacity of loblolly pine roots was measured using the depletion

16 technique (Rennenberg *et al.*, 1996; Gessler *et al.*, 1998; BassiriRad *et al.*,

17 1999). We carefully excavated terminal fine root branches of loblolly pine near

18 the soil surface. Fine root branches of loblolly pine were identified to species by

19 tracing them to coarse roots, which differed in color and texture from roots of

20 understory deciduous trees and shrubs. Root branches used in our experiments

21 were composed of roots primarily less than 2 mm in diameter, which are

22 considered most active in nutrient uptake. These excavated root branches

1   contained mycorrhizal short roots, but most of the extramatrical hyphae were

2   severed during the excavation and cleaning process.

3   During excavation, we washed roots with deionized water to remove soil

4   particles and adhering organic matter. Each root was placed in a 50-ml tube

5   containing 27 ml of nutrient solution. Nutrient solutions were prepared in the

6   laboratory, using concentrations based on data from soil solution collected by

7   low-tension lysimeters at 15 cm (Markewitz *et al.*, 1998). Nutrient concentrations

8   were intended to represent a range of values from one to ten times the average

9   soil solution concentrations (1X, 3X, 5X, 7X, 10X). The actual concentrations

10   differed somewhat from the predicted values. In our analyses, we used the actual

11   concentrations to which the root branches were exposed (Table 1). Tubes

12   containing nutrient solution but no roots were used as controls to determine the

13   amount of evaporation and contamination that occurred during the experiments.

14   Tubes with and without roots were covered with parafilm to reduce evaporation

15   and were aerated with ambient air using a battery-powered pump system

16   comprised of tubing linked to pipette tips inserted in the tubes. Nutrient solutions

17   were collected after specified intervals, and the volume of solution was measured

18   in order to correct for uptake or evaporation of water. Solutions were filtered on

19   site using syringe filters with 0.4 μm membrane filters. Samples were

20   transported in coolers to the laboratory where they were frozen until analysis.

21   After the uptake experiments were completed, the portion of the root immersed in

22   the solution was severed from the tree, weighed, and stored in 50% ethanol.

23   Roots were weighed after oven-drying at 70 $^\circ$C.

1   Nitrate and NH_4^+ concentrations were determined by continuous flow

2   analyzer and autoanalyzer (model AA3; Bran and Luebbe, Norderstedt,

3   Germany). Cation (Ca^{+2}, Mg^{+2}, and K^+) concentrations were determined using

4   inductively coupled plasma emission spectroscopy (model FMA-03, Spectro

5   Analytical Instruments, Kleve, Germany). Net nutrient uptake rates were

6   calculated from the change in nutrient content of the solution (concentration

7   times volume) over the time period. Rates were expressed as a function of dry

8   weight for each root. Since we did not measure influx and efflux independently,

9   our rates are expressed as net uptake rates with positive values indicating net

10   uptake of nutrients and negative values indicating net efflux of nutrients. These

11   rates include any change in nutrient storage by microbes on the roots, which we

12   could not control, but was probably small. Contamination and evaporation were

13   also generally small; the K, NH_4^+, NO_3^-, Mg and Ca concentrations of controls

14   were not significantly different from the initial concentration in 72% of the cases.

15

16   *Temporal trends in uptake*

17   In July 2001, October 2001 and April 2002, 10 loblolly pine root branches were

18   excavated, washed with deionized water and placed in a 50-ml tube with 27 ml of

19   nutrient solution. A 15-ml tube was inserted into the 50-ml tube to displace

20   solution and increase the ratio of root surface area to solution volume.

21   To determine if uptake capacity was affected by antecedent nutrient

22   solution concentration, we initially exposed half of the roots to average soil

23   solution concentrations (1X) and the remaining roots to 10X solutions (Table 1).

1   Following the initial two-hour period, each root branch was removed and placed

2   in a new tube containing a new solution. Roots exposed to 1X were subjected

3   over successive 2-h intervals to increasing concentrations of solutions

4   (approximately 3X, 5X, 7X and 10X; Table 1). The remaining roots were

5   exposed to concentrations of solution decreasing from 10X to 1X. Since two

6   days were needed to excavate the roots and implement the treatments, we

7   exposed all roots to the same nutrient solution concentration (5X) overnight for

8   15 to 19 hours.

9

10   *Comparison of NH_4^+ and NO_3^- uptake in spring*

11   In March 2001, we excavated 12 terminal fine root branches of loblolly pine.

12   Roots were washed with deionized water and placed in a 50-ml tube with 30 ml

13   of nutrient solution. Four roots were randomly allocated to each nutrient solution

14   concentration: 1X, 5X and 20X. Five ml aliquots of solution were removed from

15   the nutrient solution after 14 h, 29 h and 100 h. Following each aliquot removal,

16   5 ml of deionized water was added to the tubes to keep volume constant and

17   provide a descending sequence of concentrations. The nutrient solutions were

18   filtered and frozen until analysis. Since the roots from this experiment were

19   accidentally discarded before analysis, we present changes in nutrient

20   concentration over time but not uptake per unit root.

21   *Statistical analysis*

22   To determine how sampling date (July, October or April) and prior treatment

23   (increasing or decreasing sequence of concentration treatments) affected uptake

1   capacity, data were analyzed using generalized linear models (SAS Institute,

2   1985) with nutrient solution concentration treatment (1X, 3X, 5X, 7X, 10X) as a

3   repeated measure. Since the 3-way interaction of sampling date, prior treatment

4   and concentration was significant for most solutes at $\alpha = 0.05$, we compared

5   temporal trends at our mid-range concentration (5X) at both 2-hour and overnight

6   time intervals. We also compared the slopes of the regression lines of uptake

7   with concentration between sampling dates and between prior treatments.

8   To determine how concentration changed with time in our March 2001

9   comparison of NH_4^+ and NO_3^-, we used time as a class variable in our model.

10   Since each root was only given one concentration in our preliminary experiment,

11   data for each concentration (1X, 5X and 20X) were analyzed separately.

12

13   **Results**

14   The analysis of our measurements of K, NH_4^+, NO_3^-, Mg and Ca uptake at five

15   different nutrient concentrations (1X, 3X, 5X, 7X, 10X) and two different

16   antecedent condition in three months of the growing season resulted in a three-

17   way interaction of concentration, antecedent condition and time of year for most

18   nutrients. We first present temporal variation in net uptake capacity at our mid-

19   range concentration (5X) to show how uptake varied with experiment duration

20   and sampling date. Next, we discuss the effects of antecedent conditions and

21   nutrient solution concentration on temporal trends of uptake capacity. Finally, we

22   compare the rates of NH_4^+ and NO_3^- uptake.

23

1   *Temporal trends of net uptake at 5X in 2-hour experiments*

2   We predicted that net uptake capacity of all nutrients would be highest in

3   April, when plants at this location are most physiologically active, and lowest in

4   October.   Surprisingly, net uptake of K and NH_4^+ at the 5X concentration was

5   similar in April and October (Figure 1). In July, efflux of K and NH_4^+ exceeded

6   uptake. Net uptake of Mg and Ca was positive at all sampling dates, and the

7   rates were statistically indistinguishable across dates. High variation among

8   roots precluded detection of differences smaller than 4 µmol gdwt^{-1} h^{-1} for Mg

9   and 7 µmol gdwt^{-1} h^{-1} for Ca.

10

11   *Temporal trends of net uptake capacity at 5X in overnight experiments*

12   We also examined how net uptake of K, NH_4^+, NO_3^-, Mg and Ca differed in July,

13   October and April when roots were left in solution for 15-19 h. The comparisons

14   of uptake across the three seasonal times were similar between 2 h and

15   overnight experiments, except for NH_4^+ (Figure 1). Ammonium uptake did not

16   vary by sampling date in the overnight experiments, because nearly all of the

17   NH_4 (92%) was taken up in the first two hours.

18   Nitrate was measured only in the overnight experiments since uptake

19   rates were too low to be detectable in 2 h. As expected, net nitrate uptake was

20   highest in April (Figure 1). In July and October, efflux of NO_3^- exceeded uptake.

21   Uptake of Mg and Ca was higher in the 2h than overnight experiments, not

22   because there was not enough solute remaining in the tubes to sustain uptake at

23   the 2-hr rate as observed with NH_4^+. In fact, the final concentration of K, Mg, Ca

1   in the tubes was similar in the overnight (averaging 241µM K, 82 µM Mg and 85

2   µM Ca) and the 2h experiments (233 µM K, 75 µM Mg and 70 µM Ca). The

3   average rate of net uptake (µmol gdwt^{-1} h^{-1}) is thus much lower for the overnight

4   experiments although the net amount of uptake (µmol gdwt^{-1}) was similar. This

5   suggests that net uptake was positive for no more than 2 hours, after which efflux

6   equaled influx.

7

8   *Effects of antecedent conditions on temporal trends of net uptake capacity*

9   To examine how antecedent conditions affect net uptake, we exposed our roots

10   to either increasing (1X to 10X) or decreasing (10X to 1X) nutrient solution

11   concentrations. We found that the sequence of treatments affected uptake rates

12   of NH_4^+, Mg and Ca in July but not in October or April. In July, antecedent

13   conditions affected the relationship between uptake and concentration for NH_4^+

14   ($p = 0.03$), Mg ($p < 0.0001$) and Ca ($p = 0.01$). In contrast to our prediction, roots

15   exposed to initially high concentrations ("decreasing") had higher uptake of NH_4^+,

16   Mg and Ca than roots exposed to lower concentrations first ("increasing", Figure

17   2). In July, K uptake was relatively constant across concentration and was

18   unaffected by antecedent nutrient concentrations.

19   In October and April, net uptake was not affected by antecedent root

20   conditions, but the pattern of uptake with concentration differed between these

21   two sampling times. Although we predicted that uptake would increase with

22   concentration, uptake of Ca, Mg, K and NH_4^+ was relatively constant across

23   concentration in October (Figure 2). In April, when plant roots may be more

1   physiologically active, Ca, Mg, K and NH_4^+ uptake significantly increased with

2   nutrient solution concentration (R^2= 0.56, 0.75, 0.36, and 0.38 respectively).

3

4   *Timing of net NO_3^- and NH_4^+ uptake*

5   We predicted that net NH_4^+ uptake of loblolly pine roots would exceed net NO_3^-

6   uptake in our March 2001 experiment. During the first 14 hours, NH_4^+ was

7   rapidly depleted at all three initial concentrations (p = <0.0001 at 1X, p = <0.0001

8   at 5X, p = 0.03 at 20X; Figure 3). At 5X and 1X, NH_4^+ concentrations dropped by

9   98% and 96% after only 14 h. Even at the highest concentration (20X), average

10   NH_4^+ concentration dropped by 60% after 14 h, and solutions were 97% depleted

11   at 100 h.

12   In contrast, net NO_3^- uptake was delayed. In the first 14 h, there was no

13   significant net uptake, and nitrate efflux exceeded nitrate uptake at all

14   concentrations (Figure 3). Between 14 and 100 hrs, depletion of at least 89%

15   occurred at all three concentrations (p = 0.02 at 1X, p = 0.04 at 5X, p =0.004 at

16   20X), indicating that net nitrate uptake capacity was induced after an initial lag

17   period.

18

19   **Discussion**

20   Nutrient accumulation in trees is generally thought to vary seasonally, with

21   maximum uptake of nitrogen in summer (Millard and Proe, 1992; Millard, 1994;

22   Gessler *et al.*, 1998) and of cations (K, Mg and Ca) in spring (Stassen and

23   Stadler, 1988). We measured uptake capacity, rather than uptake at ambient

1   conditions, in our study with loblolly pine at Calhoun Experimental Forest to

2   determine whether changes in uptake capacity might contribute to changes in

3   nutrient accumulation over time. We found that uptake capacity did not follow the

4   seasonal pattern expected of plant nutrient accumulation.

5   In general, NH_4^+ and NO_3^- uptake capacity in mature loblolly at Calhoun

6   was lower in July than April. In contrast, N accumulation was lower in spring

7   than summer in sand-grown *Picea sitchensis* (Millard and Proe, 1992) and field-

8   grown *P. sitchensis* and *Acer pseudoplatanus* (Millard, 1994). In those studies,

9   remobilized N apparently satisfied plant demand for N in spring, while uptake

10 supplied N in summer. Although different species were used, these studies

11 demonstrate that seasonal patterns of nutrient uptake capacity may differ from

12 those of nutrient accumulation.

13   Temporal patterns in uptake have sometimes been attributed to variation

14 in soil temperature. Intact roots of subalpine beech and spruce had their highest

15 NH_4^+ uptake in July and uptake was significantly correlated with soil temperature

16 (Gessler *et al.*, 1998). In contrast, we observed low uptake capacity in July when

17 air temperature was highest. Uptake capacity may have been limited by soil

18 water availability in July, though the roots were in nutrient solutions when we

19 measured uptake. Since we measured uptake at ambient temperatures, we

20 cannot address how temperature affects uptake capacity independent of other

21 seasonally varying environmental factors.

22   Nitrate uptake of mature loblolly pine was negligible in the 2-h experiments

23 and extremely low in the overnight experiments, indicating that short-term

1   measurements of NO_3^- uptake may not be providing reliable estimates of uptake

2   capacity in mature trees. In a previous study, nitrate uptake by subalpine spruce

3   and beech was generally not detectable if measured over a 4-h time interval

4   (Gessler *et al.*, 1998). Since NO_3^- concentrations in soil solution ranged from

5   only 3 to 10.1 μmol_c L^{-1} NO_3-N in the top 15cm at Calhoun (Markewitz *et al.*,

6   1998), nitrate reductase activity may have been low in the loblolly pine roots (Li

7   and Gresshoff, 1990). The nitrate present in the nutrient solution should increase

8   nitrogen reductase activity, but induction and transport to the roots may take

9   several hours. In our longest experiment, we induced NO_3^- uptake sometime

10 between 14 and 100 h (Figure 3).

11   Since NH_4^+ can inhibit NO_3^- influx (Lee and Drew, 1989) and induce NO_3^-

12 efflux (Dean-Drummond and Glass, 1983), the low rates of net NO_3^- uptake we

13 observed may have been affected by NH_4^+ in the nutrient solution. Ammonium

14 uptake occurred at a much faster rate than NO_3^-, a result that agrees with

15 previous studies showing a preference for NH_4^+ by conifers (Cole, 1981;

16 Rygiewicz and Bledsoe, 1986; Gijsman, 1990; Marschner *et al.*, 1991;

17 BassiriRad *et al.*, 1997; Gessler *et al.*, 1998). The higher capacity for uptake of

18 NH_4^+ than NO_3^- may be an adaptation to the greater availability of NH_4^+ in the

19 forest floor at Calhoun (Markewitz *et al.*, 1998) or it may reflect the lower

20 energetic costs of uptake and assimilation of NH_4^+ relative to NO_3^- (Bloom *et al.*,

21 1992).

22   Potassium uptake capacity was highest in the spring, consistent with

23 observations of K accumulation in peach trees (Stassen and Stadler, 1988).

1   Seasonal changes in K uptake have been studied primarily using young fruit

2   trees (Gries *et al.*, 1993; Picchioni *et al.*, 1997) and agricultural crops (Clark and

3   Smith, 1992; Hocking, 1994); more studies are needed to assess seasonal

4   changes in uptake by mature forest trees.

5   Although K efflux of roots may indicate hypoxic conditions (Escamilla and

6   Comerford, 1998), all roots were aerated in our study. We observed net K efflux

7   by loblolly pine in July but not October or April. In a previous study, however,

8   intact slash pine roots took up K in July (Escamilla and Comerford, 1998).

9   Differences in starting concentrations do not explain the differences in uptake.

10   We observed net K efflux by loblolly pine at concentrations of 48 µM while slash

11   pine roots took up K at 25.6 µM (Escamilla and Comerford, 1998).

12   Root disturbance may be partly responsible for the K efflux in our study.

13   Slash pine roots were excavated six months prior to measurement by Escamilla

14   and Comerford (1998), while the plant roots in our study were excavated just

15   prior to analysis. In previous studies, root disturbance stimulated NO_3^- efflux

16   (Aslam *et al.*, 1996) and decreased uptake of K, NH_4^+ and NO_3^- (Bloom and

17   Caldwell, 1988). Exposing roots to different pretreatments designed to minimize

18   disturbance, however, did not decrease cation efflux by mature sugar maple, red

19   pine and Norway spruce (McFarlane and Yanai, In press).

20   There were no statistically significant temporal variations in the capacity

21   for uptake of Ca and Mg by mature loblolly pine at Calhoun. No studies to date

22   have examined seasonal trends in Ca and Mg uptake by roots, although studies

1   of seasonal changes in foliar concentrations indicate that plant demand is higher

2   in spring and summer than in fall (Gries *et al.*, 1993).

3   Nutrient uptake capacity is sometimes observed to follow saturation

4   kinetics (Epstein, 1976). We found that the relationship between uptake and

5   concentration, when there was one, was generally linear, such that uptake would

6   be best described with a slope and intercept. Our most important finding,

7   however, was that uptake kinetics were not constant over time, such that using a

8   relationship measured at one point in time could fail to predict rates at another

9   point in time.

10   Experimental conditions, such as nutrient solution concentration and

11   experiment length, may affect uptake rates observed in the field. In our study,

12   NH_4^+ uptake (-3.6 µmol gdwt^{-1} h^{-1}) was much lower than previously reported for

13   intact roots of loblolly pine in August (18.8 µmol gdwt^{-1} h^{-1}) (BassiriRad *et al.*,

14   1997). That study used higher concentrations (1700 µmol L^{-1}) than ours (5 - 92

15   µmol L^{-1}) and a longer exposure time (24 h, compared to our 2 h).

16   In our study, net uptake rates of NH_4^+, Mg and Ca declined with exposure

17   times. Declining NH_4^+ uptake rates over time were associated with depletion of

18   NH_4^+ in the applied solution. In contrast, Mg and Ca concentrations were similar

19   at the end of the 2h as compared to the overnight experiments, suggesting that

20   net uptake was negligible after the first 2 h. Declining uptake rates of Mg and Ca

21   could be associated with the duration of the experiments or with attaining a

22   concentration below which net uptake was not possible. In any case, it can be

1   misleading to report uptake rates as instantaneous rates, when the duration of

2   experiments has such significant effects on uptake.

3   Species may differ in their uptake rates, even when experimental

4   conditions are similar. In July, spruce and beech roots had higher average NH_4^+

5   uptake rates (1.4, 0.9 µmol gfw^{-1} h^{-1}) (Gessler *et al.*, 1998) than loblolly pine in

6   our study (-4.0 µmol gfw^{-1} h^{-1}) when trees were exposed to similar NH_4^+

7   concentrations (approximately 55 µM). In April, however, our loblolly pine had

8   higher uptake rates (2.9 µmol gfw^{-1} h^{-1}) than spruce (0.02 µmol gfw^{-1} h^{-1}), and

9   beech (0.3 µmol gfw^{-1} h^{-1}) Gessler *et al.* (1998).

10   This is the first study to show that the effects of antecedent conditions on

11   uptake capacity vary temporally. Antecedent nutrient conditions influenced root

12   uptake in July but not in October or April. In July, loblolly pine roots exposed to

13   high initial concentrations had high NH_4^+, Mg and Ca uptake rates, indicating that

14   the roots may have been exposed to low levels of these elements in the soil

15   (Lee, 1993). Roots exposed to successively higher concentrations had lower

16   uptake at high concentrations, due perhaps to saturation of exchange sites at the

17   root surface (Dean-Drummond, 1982; Siddiqi *et al.*, 1990).

18   The technique used in this study to obtain intact roots required removal of

19   the roots from the surrounding soil, which disrupts the extramatrical hyphae of

20   mycorrhizae. Since ectomycorrhizae play an important role in nutrient uptake of

21   loblolly pine seedlings (Smith and Read, 1997), additional studies are needed to

22   assess the importance of mycorrhizae on the temporal pattern of nutrient uptake

23   capacity of mature trees.

1

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13

1 **Figure Legends**

2 Figure 1. Uptake of K, NH_4^+, NO_3^-, Mg, and Ca by intact roots of mature loblolly

3 trees, expressed on a per dry-weight of root per hour basis, in July 2001, October

4 2001 and April 2002 at concentrations five times higher than lysimeter soil

5 solution concentrations (5X). Roots were left in solution for a 2-h interval and

6 overnight (15-19 h). In the case of NO_3^-, only overnight data is presented. Error

7 bars show standard errors of the mean (n=10). Means with different letters differ

8 significantly at $\alpha \leq 0.05$.

9

10 Figure 2. Uptake of K, NH_4^+, Mg and Ca on a dry-weight basis by intact roots of

11 mature loblolly trees as a function of nutrient solution concentration in July 2001,

12 October 2001 and April 2002.   Measurements were conducted over 2 h

13 intervals. In July, uptake was significantly different when roots were exposed to

14 successively higher concentrations (increasing, - - - - -) than successively lower

15 (decreasing, ⎯⎯) concentrations. In October and April, there was no

16 difference in uptake based on antecedent concentrations and therefore only lines

17 for regressions that are significantly different from zero ($p \leq 0.05$) are shown

18 through all the data (n=10). None of the regressions for October were

19 statistically significant.

20

21 Figure 3. Time course of NH_4^+ and NO_3^- concentration over a 100-h period using

22 intact roots of mature loblolly pine trees in March 2001 exposed to three initial

23 concentrations (1X, 5X, 20X). Error bars show standard errors of the mean

1 (n=10). Concentrations were diluted by additions of distilled water at each

2 sampling time.

3

1   Table 1. Average concentration (1X to 10X) to which the roots were exposed in

2   July 2001, October 2001 and April 2002. Concentrations were based on soil

3   solution data at a depth of 15 cm at Calhoun Experimental Forest, SC (Target

4   1X; Markewitz *et al.*, 1998). Values are expressed as the average concentration

5   (µM) and standard error (n=30, except for Target 1X where n=8).

6

Conc.	Average concentration (± SE) (µM)			
	K	NH_4^+	Mg	Ca
1X	46 ± 2	10 ± 1	19 ± 5	28 ± 5
3X	133 ± 9	31 ± 6	60 ± 13	88 ± 30
5X	223 ± 16	40 ± 1	100 ± 19	185 ± 27
7X	350 ± 10	60 ± 6	156 ± 20	156 ± 45
10X	408 ± 24	78 ±12	360 ± 32	360 ± 71
Target 1X	39 ± 4	8 ± 4	21 ± 2	33 ± 3

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