

Nitrogen Effects on Herbage Nitrogen Use and Nutritive Value in a Meadow and Loblolly Pine Alley

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ABSTRACT

Herbage response to N is poorly understood in alley cropping systems. Our objective was to determine site specific effects of N on herbage N use and nutritive value in separate experiments conducted in a meadow and a loblolly pine (*Pinus taeda* L.) alley (995 trees ha⁻¹) near Booneville, AR. Tall fescue (*Festuca arundinacea* Schreb.) was the predominant herbage species. Fertilizer N was broadcast as split-applications at six rates (100 kg ha⁻¹ increments from 0 to 500 kg ha⁻¹ yr⁻¹). The proportion of total herbage as tall fescue was favored at rates \geq 200 kg N ha⁻¹, but these rates increased concentrations of herbage NO₃⁻-N in the pine alley to potentially harmful levels for grazing ruminants (>2300 μ g NO₃⁻-N g⁻¹). Cumulative herbage N use efficiency (CNUE) was 23 and 10 kg dry weight per kilogram supplied N for the meadow and pine alley, respectively. Cumulative N acquisition efficiency (CNAE), not cumulative N conversion efficiency (CNCE), appeared to be the primary driver of low CNUE in the pine alley. The apparent increase in crude protein (CP) in pine alley vs. meadow herbage appeared to be a mechanistic response to decreased specific leaf weight. Only maintenance levels (≤ 100 kg ha⁻¹) of N fertilization should be applied to pine alley herbage given the low CNUE, and risk of NO₃⁻-N toxicity to ruminant livestock at higher N rates.

ALLEY CROPPING SYSTEMS can be designed for the joint production of herbage, livestock, and wood fiber. Substantial herbage can be produced in alleys during the tree rotation (Fribourg et al., 1989; Lewis and Pearson, 1987). Depending on system components and design, however, the subcanopy environment of alley cropping systems can constrain herbage production because of low solar irradiance, low soil water (Burner and MacKown, 2005), and competition for soil N (Campbell et al., 1994) and other soil nutrients (Lehmann et al., 1999). Reduction in herbage productivity could be caused in part by altered anatomy and physiology in the tree understory micro-environment (Devkota and Kemp, 1999). Shade-grown tall fescue yields less total dry matter per plant, primarily due to fewer tillers per plant, and has lower specific leaf weight (SLW), lower CO₂ exchange rate (CER), and more leaf lamina air space than plants at high irradiance (Allard et al., 1991a, 1991b). Similarly, tall fescue adapts to water stress by reducing plant size and growth rate, decreasing evaporative surface area, increasing root/shoot ratio, and reducing stomatal conductance (Assuero et al., 2002).

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Loblolly pine has rapid growth on sites with low inherent soil fertility and minimal fertilizer inputs (Schultz, 1997). The success of loblolly pine-based alley cropping systems depends on the selection and management of appropriate shade tolerant herbage species for optimal productivity and sustainability (Devkota and Kemp, 1999). Tall fescue is tolerant to growth at low irradiance (Burner, 2003; Clason and Sharrow, 2000; Lin et al., 1999) and water deficit (Wilman et al., 1998). Cumulative herbage yield (CHY) was 40% of that in an open meadow even when solar irradiance was only 12 to 37% of that in the meadow (Burner and MacKown, 2005). Tall fescue usually exhibits a positive yield response to increasing N fertilization in conventional pastures (Wilkinson and Mays, 1979). There has been relatively little research on N response of cool season grasses in alley cropping systems. Tall fescue responded to supplemental fertilization in a loblolly pine alley on marginal land (Brauer et al., 2004), where its production appeared to be unsustainable without fertilization (Burner and Brauer, 2003; Burner and MacKown, 2005).

Herbage in conventional pasture and alley cropping systems is managed for microclimate and spatial differences inherent to these systems, but managers have scarce data on which to base their decisions. Herbage in a pine alley recovered only a third as much of the cumulative fertilizer N as in a meadow (Burner and MacKown, 2005), demonstrating that production environment affects N response. This is important because N fertilizers cost US\$0.50 to 1.91 kg⁻¹ N (USDA-NASS, 2004). Economic efficiency, herbage and livestock production, and tree growth components need to be considered for effective N management. Our objective was to determine effects of applied N on the relative abundance of tall fescue herbage, CNUE and its components CNCE and CNAE, and nutritive value in a meadow without trees and a loblolly pine alley, to support fertilizer management decisions for alley crop production.

MATERIALS AND METHODS

Two experiments were conducted near Booneville, AR, one in a tall fescue meadow and one in a loblolly pine stand with a predominantly tall fescue understory. Experimental sites were about 1.7 km apart on the same soil type, a Leadvale silt loam (fine-silty, siliceous, semiactive, thermic Typic Fragiu-dult). Swards were at least 10 yr old.

Abbreviations: CER, CO₂ exchange rate; CHY, cumulative herbage yield; CMN, cumulative mineralized soil N; CNAE, cumulative herbage N acquisition efficiency; CNCE, cumulative herbage N conversion efficiency; CNS, cumulative N supply; CNUE, cumulative herbage N use efficiency; CNY, cumulative N yield; CP, crude protein; HSD, honest significant difference; IVDMD, in vitro dry matter digestibility; NUE, N use efficiency; PAR, photosynthetically active radiation; SLW, specific leaf weight.

One-yr-old loblolly pine seedlings were planted at 2.4-m spacing in rows 3.6 m wide oriented east-west in spring 1994. There were 995 trees ha⁻¹ in 2002. Herbaceous vegetation was a complex mixture of cool- and warm-season grasses and forbs (Burner and Brauer, 2003; Burner and MacKown, 2005).

In 2002 and 2003, NH₄NO₃ was broadcast applied in split applications (one-third rate in March, May, and September) at 0, 100, 200, 300, 400, and 500 kg N ha⁻¹ yr⁻¹. The N fertilizer treatments were applied to each experiment in a randomized complete block design with three replicates. Treatment plots measured 2.5 by 6 m and were separated by grass covered buffers \geq 1.5 m. In the pine alley, plots were positioned between tree rows, so <50% of the root zone of any tree was included within the plot boundary. Each replicate was separated by at least one unfertilized 3.6-m-wide pine alley (Fig. 1).

The proportion of tall fescue, tall fescue SLW, and tall fescue CER were measured for each plot a few days before each harvest date, except that CER was not measured in June 2003. The proportion of tall fescue was a visual estimate of relative dry mass contributed to CHY. The SLW was calculated from the surface area (one-sided) and dry mass of a 10-cm segment of the lamina of eight most recently fully expanded leaves from each plot. The CER was measured on clear, sunny days between 0900 and 1100 h CST. A leaf from two plants that had been fully illuminated \geq 15 min before sampling was measured in each plot. Instrument calibration and sampling procedure were described previously (Burner, 2003).

Harvests to measure total herbage dry matter yield were conducted on 2 May and 17 Oct. 2002 and 6 May, 23 June, and 15 Oct. 2003 as previously reported (Burner and MacKown, 2005).

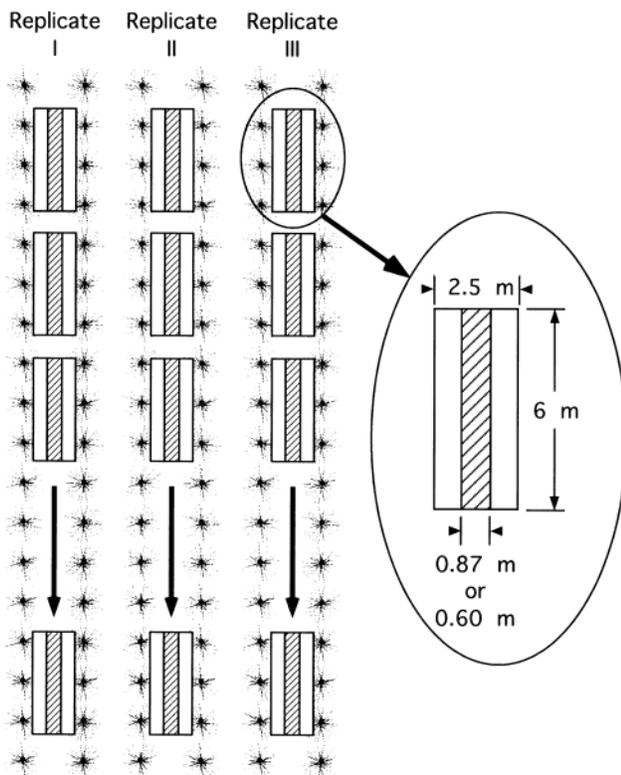


Fig. 1. Schematic drawing of plots in loblolly pine alleys. Trees (dots) were spaced 2.4 m within rows and 3.6 m between rows. One or more buffer alleys separated replicates. The N treatments (rectangles) assigned to plots were randomized within replicates. Herbage and soil samples were collected from the central region (striped) of each plot. Only three of the six plots are shown; heavy downward arrows represent plots not shown.

Samples for nutritive value were collected from harvested herbage, dried (60°C), and ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) to pass a 1-mm screen. After each harvest date and before N application, two soil cores (6.4-cm diameter by 60 cm long) were collected with a Giddings hydraulic probe (Giddings Machine Co., Ft. Collins, CO) from each plot, sectioned into three layers (0–10, 10–30, and 30–60 cm depth), and combined by depth. Soil samples were prepared and analyzed for mineral N (NH₄ and NO₃) as described previously (Burner and MacKown, 2005). Herbage total N was determined by Dumas combustion (Leco FP428, Leco Corp., St. Joseph, MI). Herbage CP was 6.25(total N). Samples of herbage were extracted with hot water (97°C, 1 h) and the extracted NO₃⁻-N was measured by the Cd reduction method (Mulvaney, 1996) using a flow injection analysis instrument (FI Astar 5010 Analyzer, Foss Tecator, Höganäs, Sweden). Trees were not sampled for foliar yield or N. Herbage in vitro dry matter digestibility (IVDMD) was determined using the procedure of Goering and Van Soest (1971), modified for the Ankom Daisy II fiber analyzer no. F200 (ANKOM Technology Corp., Fairport, NY).

There may have been some N carryover between successive harvest dates and years (Vanotti and Bundy, 1994), so cumulative effects were calculated (Staley et al., 1991). Nitrogen use components were calculated by the following equations:

$$\text{Cumulative N yield (CNY, kg ha}^{-1}\text{)} = (\text{Cumulative herbage yield, kg ha}^{-1}\text{})(\text{herbage N, g kg}^{-1}\text{})(0.001 \text{ kg g}^{-1})$$

Cumulative herbage yield was the sum of herbage yield for each plot at successive harvest dates for 2 yr.

$$\text{Cumulative mineralized soil N (CMN, kg ha}^{-1}\text{)} = \text{soil mineral N (kg ha}^{-1}\text{)} + \text{CNY at N}_0 \text{ (kg ha}^{-1}\text{)}$$

Soil mineral N was determined for plots receiving no N fertilization (N₀), and summed across successive harvest dates. Amounts of soil mineral N were based on an average bulk density of 1.47 g cm⁻³ for a 0- to 50-cm depth profile of a Leadvale silt loam (Buell et al., 2004).

$$\text{Cumulative N supply (CNS, kg ha}^{-1}\text{)} = \text{CMN at N}_0 \text{ (kg ha}^{-1}\text{)} + \text{Cumulative fertilizer N (CFN, kg ha}^{-1}\text{)}$$

Cumulative fertilizer N was the sum of fertilizer N applied before each harvest date.

$$\text{CNCE} = \text{CHY (kg ha}^{-1}\text{)}/\text{CNY (kg ha}^{-1}\text{)}$$

$$\text{CNAE} = \text{CNY (kg ha}^{-1}\text{)}/\text{CNS (kg ha}^{-1}\text{)}$$

$$\text{CNUE} = (\text{CNCE})(\text{CNAE})$$

Cumulative NUE and its components were calculated at each harvest date for plots receiving N fertilizer (Moll et al., 1982).

Each experiment was analyzed using a mixed linear model, PROC MIXED (Littell et al., 1996; SAS Institute, 1998) for analysis of variance of tall fescue composition, CNCE, CNAE, CNUE, SLW, CER, and nutritive value. Fixed effects were harvest date (4 df), N rate (5 df), and the interaction (20 df), except that CER had 3, 5, and 15 df, and CNCE, CNAE, and CNUE had 4, 4, and 16 df, respectively. Replication (2 df) and its interactions with fixed effects were random. Degrees of freedom were approximated by Satterthwaite's method (SAS Institute, 1998). Fixed effects were tested against residual error, which had 60 df except for CER (120 df), and CNCE, CNAE, and CNUE (32 df). Means were considered different at $P \leq 0.05$ using Tukey's honest significant difference (HSD) (SAS Institute,

1998). Responses of CNCE, CNAE, and CNUE to yearly fertilizer N were analyzed by linear regression using the Fit Y by X Platform of JMP statistical software (SAS Institute, 2002).

RESULTS AND DISCUSSION

For each experiment, the proportion of tall fescue was greater in May ($\geq 92\%$) than at subsequent harvest dates ($\leq 78\%$). At the final harvest date, the proportion of tall fescue was 50% in the meadow and 78% in the pine alley, and cumulative tall fescue yield was 82% (meadow) and 85% (pine alley) of CHY (Burner and MacKown, 2005). The percentage of tall fescue was not affected ($P > 0.17$) by N fertilization at either May harvest date ($\geq 92\%$), but at other harvest dates the proportion of tall fescue usually followed the order $0 \text{ kg N ha}^{-1} (\leq 38\%) < 100 \text{ kg N ha}^{-1} (\leq 66\%) < \text{all other N rates} (\leq 90\%)$. The data support our previous observation (Burner and Brauer, 2003) that tall fescue may not be sustainable in unfertilized pine alleys. Besides tall fescue, annual bluegrass (*Poa annua* L.), broomsedge (*Andropogon virginicus* L.), and foxtail (*Setaria* spp. Beauv.) were common weedy grasses, and clover (*Trifolium* spp. L.), horsenettle (*Solanum carolinense* L.), persimmon (*Diospyros virginiana* L.), and trumpet creeper (*Campsis radicans* L. Seem.) were common weedy forbs in meadow plots. Bermudagrass [*Cynodon dactylon* (L.) Pers.], panicum (*Panicum* spp. L.), and purpletop [*Tridens flavus* (L.) Hitchc.] were common weedy grasses, and horsenettle was a common weedy forb in the pine alley.

The harvest date \times N rate interaction was significant ($P \leq 0.05$) for CNAE in both experiments, CNUE in the pine alley ($P = 0.01$), but not for CNCE in either experiment ($P \geq 0.12$). The interactions were mainly due to changes in magnitude of differences among N rate treatments at each harvest date, but N rate and harvest date main effects were consistent, so the interaction effects were ignored and only the main effects were presented. Harvest date and N rate main effects were significant in both experiments ($P \leq 0.02$). The CNCE ranged from 53 to 60 in the meadow and 46 to 58 in the pine alley (Fig. 2, A1). Similarly, CNAE ranged from 0.34 to 0.44 in the meadow and 0.18 to 0.20 in the pine alley (Fig. 2, B1). Means for CNAE were comparable to mean apparent N recovery of Zemenchik and Albrecht (2002). The CNUE ranged from about 20 to 24 in the meadow and about 9 to 10 in the pine alley (Fig. 2, C1). Means for CNUE in the meadow were consistent with previous reports for tall fescue (Lemaire et al., 2004; Staley et al., 1991) and other cool season grasses (Zemenchik and Albrecht, 2002). However, species composition might have confounded CNUE and its components relative to that expected for tall fescue. The primary contributor to low CNUE in the pine alley appeared to be CNAE, not CNCE. Responses to N treatment (averaged across harvest dates) generally decreased with increasing N rate (Fig. 2, A2, B2, and C2), except for CNAE in the meadow (Fig. 2B2). The CNCE in both experiments converged at the high N rate (Fig. 2, A2). The y intercepts for CNAE were 0.466 and 0.259 in the meadow and pine

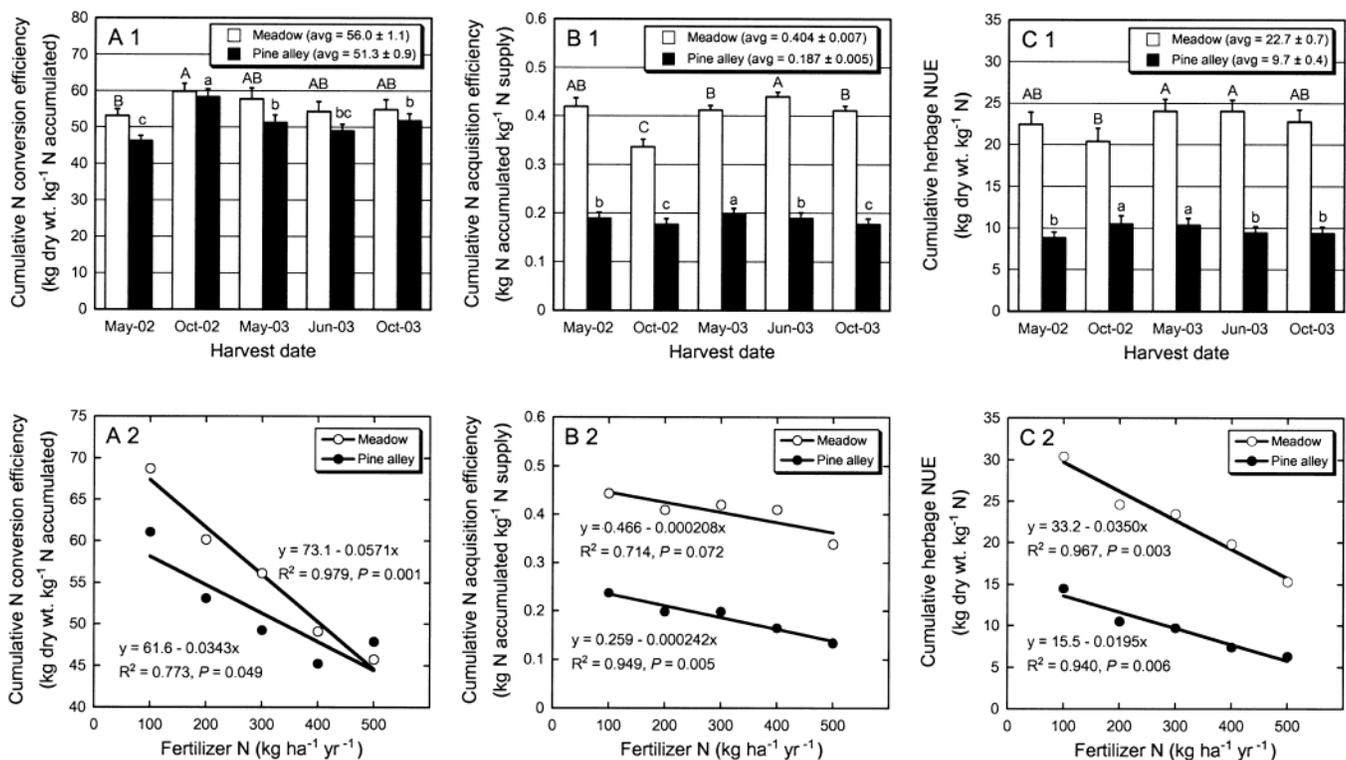


Fig. 2. Harvest date and N rate treatment effects on cumulative herbage N conversion efficiency (A1 and A2), N acquisition efficiency (B1 and B2), and cumulative N use efficiency (NUE) (C1 and C2) in a meadow and pine alley. Bars with the same letter (capitals for meadow and lowercase for pine alley) are undifferentiated ($P \leq 0.05$) by Tukey's honest significant difference (HSD). Small vertical bars represent SE.

alley, respectively (Fig. 2, B2), and those for CNUE were 33.2 and 15.5 for the meadow and pine alley, respectively (Fig. 2, C2). The decline in CNCE and CNAE with increasing fertilizer N is expected (Lemaire et al., 2004) and represents progressively increasing N concentration (see below) without increasing growth (luxury consumption). The low CNAE for pine alley herbage may be partially the consequence of competition for N with the trees. However, water stress and low solar irradiance in the alleys (Burner and MacKown, 2005) probably decreased the supply of assimilate for energy needed to drive nutrient accumulation and assimilation.

The harvest date \times N rate interaction affected tall fescue SLW in the meadow ($P = 0.01$) but not the pine alley ($P = 0.34$). The interaction appeared to be caused by changes in ranking across harvest dates in the meadow (Fig. 3). The SLW usually was greater at 0 or 100 kg N ha⁻¹ compared to higher N rates in May 2002 through June 2003, but there was no difference in SLW among N rates in October 2003. Values were comparable to tall fescue grown at high (unshaded, 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and low (shaded, 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$) irradiance, respectively (Allard et al., 1991a). At any given harvest date or N rate, SLW was 55 to 74% greater in the meadow than the pine alley (data not shown). Daily photosynthetically active radiation (PAR) for the pine alley (7 mol m⁻² d⁻¹) was 22% of that in the meadow (32 mol m⁻² d⁻¹) (Burner and MacKown, 2005). Artificial shade induces changes in leaf anatomy, including SLW, which has a strong influence on leaf gas exchange properties (Allard et al., 1991a).

Mean CER was influenced only by harvest date in each experiment. Mean CER of tall fescue was 12.2 and 8.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the meadow and pine alley, respectively. These values were about half that reported for

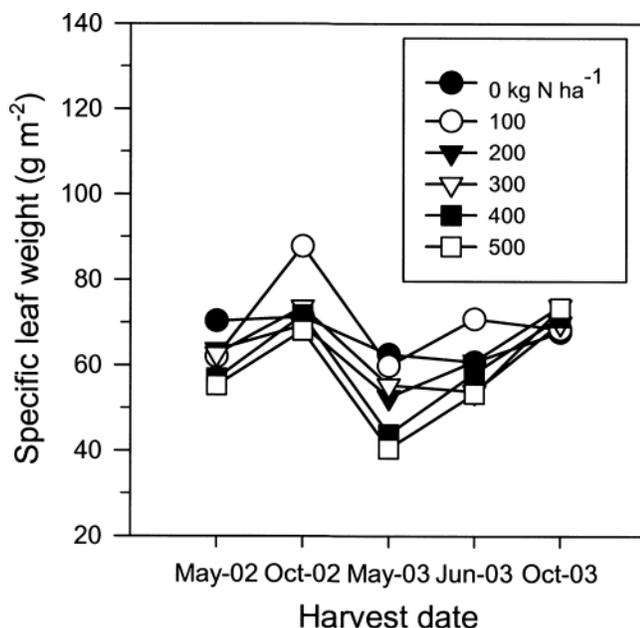


Fig. 3. Effect of the harvest date \times N rate interaction on specific leaf weight of tall fescue in the meadow. Standard error bars not shown for clarity.

tall fescue in a controlled-environment chamber (Allard et al., 1991b). Photosynthetic capacity of shade grown plants tends to be less responsive to available irradiance than unshaded plants (Givnish, 1988). However, CER and PAR were confounded in each experiment. Mean PAR received at the leaf during CER sampling was 1430 and 1160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the meadow and pine alley, respectively, indicating we were unsuccessful in completely avoiding transient shading of leaves in the pine alley. However, leaf irradiance closely approached (meadow) or met (pine alley) CO₂ saturation concentrations (Allard et al., 1991b), indicating that confounding effects might have been negligible. Five tall fescue plants selected at random from the meadow and pine alley did not differ in CER when grown in the greenhouse (D.M. Burner, unpublished data, 2003), demonstrating that low photosynthetic capacity of tall fescue from the pine alley was reversible.

High N rates combined with climatic conditions that reduce plant growth can foster NO₃⁻-N accumulation by plants (Strickland et al., 2003). Herbage NO₃⁻-N concentrations of 700 to 2300 $\mu\text{g NO}_3^- \text{N g}^{-1}$ can impair reproductive performance of grazing ruminants, while those >2300 $\mu\text{g NO}_3^- \text{N g}^{-1}$ can cause acute toxicity (Strickland et al., 2003). The harvest date \times N rate interaction was significant for NO₃⁻-N. Herbage from the meadow presented little risk of NO₃⁻-N toxicity, except at N rates ≥ 400 kg ha⁻¹ yr⁻¹ in June 2003 (Fig. 4A). Conversely, potentially toxic concentrations of herbage NO₃⁻-N were found in the pine alley when N fertilization was ≥ 400 kg ha⁻¹ yr⁻¹ (May 2003) or ≥ 200 kg ha⁻¹ yr⁻¹ (June 2003). Concentrations were comparable to those of mixed grass-legume herbage in a pine woodland, which had 2500 to 3500 $\mu\text{g NO}_3^- \text{N g}^{-1}$ at 20 and 50% available light, respectively (Neel et al., 2001). In the pine alley, herbage concentrations of NO₃⁻-N were consistently safe only after N application in September.

The harvest date \times N rate interaction was significant for CP. The CP concentrations of herbage in May and June usually were greater than in October (Fig. 4B), perhaps reflecting the seasonal shift in proportions of cool and warm season grasses or plant maturity. The CP tended to increase with N rate in both experiments, although meadow herbage was relatively unresponsive in May 2003, and pine alley herbage was relatively unresponsive in October 2003. Mean CP was 107 and 120 g kg⁻¹ in the meadow and pine alley, respectively. These concentrations met or exceeded the minimum CP requirements for most stocker cattle, replacement heifers, and beef cows on tall fescue hay (National Research Council, 1996).

The CP concentration of herbage usually is greater in pine-shaded than unshaded sites (Burner, 2003; Burner and Brauer, 2003). It is unclear which, if any, nitrogenous constituents other than NO₃⁻-N might be selectively increased in shade-grown herbage. However, the increased CP concentration of herbage with fertilizer N probably was not due to increased NO₃⁻-N, because this was a relatively small fraction of total N. Further, concentrations of rubisco, soluble proteins, and chlorophyll are either lower or unchanged in shaded vs. unshaded

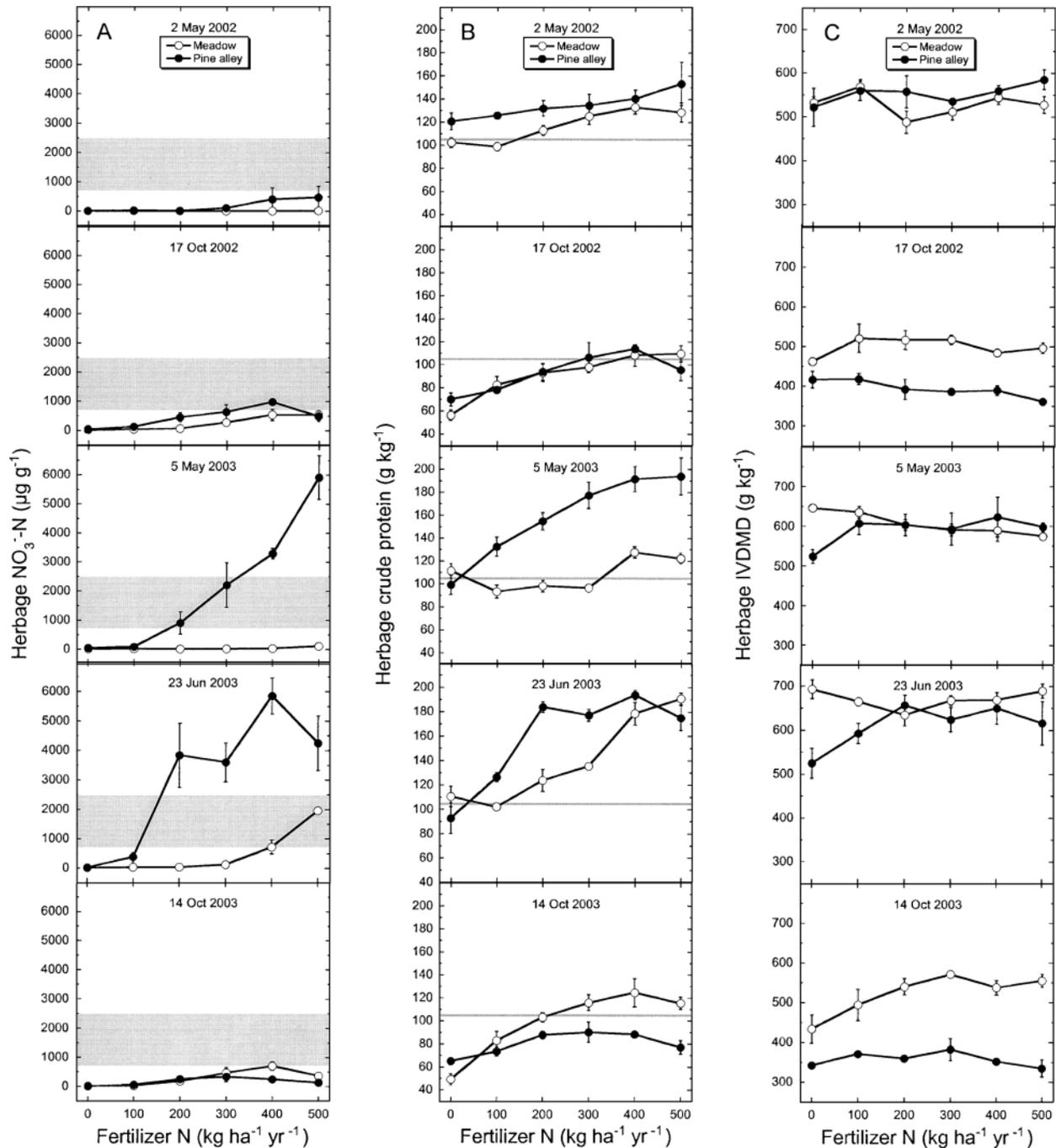


Fig. 4. (A) Changes in NO_3^- -N, (B) crude protein, and (C) in vitro dry matter digestibility (IVDMD) of meadow and pine alley herbage sampled in May 2002 through October 2003. In A, NO_3^- -N concentrations below the gray bar do not pose a risk to grazing cattle, concentrations within the gray bar represent variable risk, and those above the bar are considered unsafe (Strickland et al., 2003). In B, the gray horizontal line at 105 g kg^{-1} represents the minimum concentration for a 450-kg beef cow 1 mo after calving (National Research Council, 1996). Small vertical bars represent SE that exceed the symbol size.

plants (Allard et al., 1991b; Givnish, 1988). Conversely, shaded leaves have decreased SLW, nonstructural carbohydrates (Burner and Belesky, 2004), and cell wall concentration (Devkota and Kemp, 1999) compared to unshaded leaves. Shaded leaves also are thinner with more air space and comparable volumes of cellular tissues as unshaded leaves (Allard et al., 1991a). Nitrogen content per unit leaf area is not affected by shade, but N concentration decreases with increasing SLW in tall fes-

cue, indicating that nitrogenous compounds are concentrated in thinned, shaded leaves (Kephart and Buxton, 1993). Thus, the apparent increased concentration in CP for shaded vs. unshaded herbage could be an artifact of CP dilution associated with decreased SLW.

The harvest date \times N rate interaction was significant for IVDMD in the meadow ($P = 0.001$), but not the pine alley ($P = 0.25$). There were few differences in meadow IVDMD across harvest dates except that IVDMD in

October 2003 was less at most N rates than in May and June 2003 (Fig. 4C). As with CP, this may be related to a seasonal shift in botanical components or plant maturity, but the effect was not detected in 2002. Mean IVDMD in the pine alley was less at 0 kg N ha⁻¹ yr⁻¹ (466 g kg⁻¹) than at other N rates (range 500–515 g kg⁻¹). Mean IVDMD was 565 g kg⁻¹ in the meadow compared to 501 g kg⁻¹ in the pine alley, which was inconsistent with the finding that tall fescue IVDMD decreases with SLW (Kephart and Buxton, 1993). Response of herbage IVDMD has been inconsistent with shading (Burner, 2003; Burner and Brauer, 2003), and in comparison to nonstructural carbohydrates (Burner and Belesky, 2004).

CONCLUSIONS

The study was conducted to better understand site specific herbage N responses to fertilizer N in separate experiments conducted in a loblolly pine alley and a meadow. Low CNAE, not CNCE, was the primary contributor to low CNUE in the pine alley. Water stress and low solar irradiance in the alleys (Burner and MacKown, 2005) could have decreased the supply of assimilate for energy needed to drive nutrient accumulation and assimilation, thereby lowering CNAE for pine alley herbage. Water stress and low solar irradiance in the alleys also could have induced adaptive herbage responses of CER, SLW, NO₃⁻-N, and CP. Meadow herbage had acceptable concentrations of NO₃⁻-N when fertilized with up to 400 kg N ha⁻¹. However, only maintenance levels (≤100 kg ha⁻¹) of N fertilization should be applied to pine alley herbage given the low CNUE, and risk of NO₃⁻-N toxicity to ruminant livestock at higher N rates. The poor herbage N responses in pine alleys should improve by applying cultural practices that reduce water stress or competition for soil nutrients, or increase solar irradiance. These management practices include use of a more N-efficient herbage species such as orchardgrass (Lemaire et al., 2004; Zemenchik and Albrecht, 2002), wider alleys, tree branch or root pruning, tree thinning, and addition of any limiting nutrient.

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