

Specific Leaf Area Along a Nitrogen Fertilization Gradient

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ABSTRACT.—Specific leaf area was measured for three dominant perennial grass species in a long term nitrogen fertilization experiment in an abandoned field in Minnesota. The specific leaf area differed among the species and increased with increasing levels of nitrogen fertilization. This increase in specific leaf area was up to 82% for *Agropyron repens* and shows that the response of specific leaf area to nitrogen fertilization can be highly plastic. Increasing specific leaf area within species, together with a species replacement, resulted in a 202% increase in leaf area index along the nitrogen gradient, whereas the biomass increased by only 57%. One-third of this increase was attributed to species replacement and two-thirds to the change in specific leaf area within species. This plastic response of specific leaf area within plant species substantially increases the aboveground competition for light along nitrogen gradients.

INTRODUCTION

Plant species dominance is partly controlled by soil fertility and nitrogen fertilization has been used to explore mechanisms and tradeoffs that underlie species competitive abilities (Aerts and Berendse, 1988; Tilman, 1988; Wilson and Tilman, 1993; Foster and Gross, 1998). Plant species differ in the way they allocate biomass and this impacts their competitive ability in relation to soil nutrient and light availability. Tilman (1988) found a shift in species along a nitrogen fertilization gradient which corresponded with a shift from below— to aboveground biomass and argued that this represented a tradeoff between competitive ability for soil nutrient resources and light. However, in addition to biomass allocation, species also differ in leaf morphology and this can also impact their competitive abilities in relation to nitrogen and light availability (Olf, 1992). Here we hypothesize that this long-term nitrogen fertilization gradient causes a shift in specific leaf area (SLA) both within species and between plant communities. SLA will, together with the observed shift in aboveground biomass allocation patterns (Tilman, 1988), increase the aboveground leaf area index (LAI), thereby intensifying aboveground competition.

Specific leaf area is the ratio of the leaf area relative to leaf mass and consists of two components: leaf thickness and density (Witkowski and Lamont, 1991). Specific leaf area varies considerably between species, individuals and within plant canopies (Shipley, 1995; Cornelissen *et al.*, 1997; Garnier *et al.*, 1997) and is correlated with leaf nitrogen concentration (Reich and Walters, 1994; Garnier *et al.*, 1997), leaf gas exchange (Gutschick, 1988; Reich and Walters, 1994), growth rate (Poorter and Remkes, 1990; Garnier, 1992; Lambers and Poorter, 1992) and leaf longevity (Reich *et al.*, 1992; Reich, 1993), traits which themselves are interrelated (Reich *et al.*, 1992; Shipley, 1995).

METHODS

This study was conducted in an old field at Cedar Creek Natural History Area which was abandoned in 1968 (Field A; Tilman, 1984, 1987). Cedar Creek is located 45 km north of

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Minneapolis, Minnesota (45°24'N, 93°12'W) and has a typical midcontinental climate with hot humid summers and cold winters. The soil is a Sartell series fine sand (Grigal *et al.*, 1974) and nitrogen is the only detectable mineral nutrient limiting plant productivity within this field (Tilman, 1984). The plots were fenced to exclude herbivores with 15 cm mesh wire extending up 1.8 m and hardware cloth was buried to a depth of 84 cm. Gophers and other small mammals were trapped and removed. Tilman (1987) details experimental design and fertilization methods. In summary, the experiment was established in 1982 and has nine fertilizer treatments with six replicates per treatment; a control and 8 different rates of NH_4NO_3 addition with the same amount of other nutrients (P, K, Ca, Mg, S and trace elements) for a total of 54 plots of 4 × 4 m. All plots have a 1 m buffer area and were laid out in a 6 by 9 grid. Rates of N addition are 0, 1, 2, 3.3, 5.3, 9.3, 16.7 and 26.7 g nitrogen/m² year. Fertilizer was applied twice each year: once in early May and once in late June.

Standing biomass was sampled by clipping a 3.0 by 0.1 m strip at the soil level in each plot in early July of 1996, when the vegetation reaches its peak standing biomass. Plant samples were taken into the laboratory and sorted to individual species and litter.

The specific leaf area (SLA, the ratio of leaf area to leaf mass) was determined for the three dominant perennial grasses: *Agropyron repens*, *Poa pratensis* and *Schizachyrium scoparium*, which contributed more than 80% of the biomass to each plot and more than 97% of the average biomass of each treatment. Three whole plants of each species were collected within the fertilized area of all plots in July of 1996 and stored for transport in plastic bags. Plants were sorted into dead leaves, stems and live leaves. Leaf area was measured the same day with a Licor leaf area meter, except for *Poa pratensis* which, because its leaves are too narrow, were scanned and digitized. Samples were subsequently dried to constant dry weight at 50 C and weighed to 0.001 g. Leaf area index (the area of leaves per area of soil surface) was calculated from the SLA and the plant biomass in each plot, with the percentage of leaves and stems estimated from the whole plants that were collected for the SLA measurements. *Poa* stems were too small to measure and SLA was calculated from the relative ratio of stems to leaves, based on the relative ratio from *Agropyron* (Fig. 1).

All statistics were done with SPSS 8.0 for Windows. The two controls, with and without micronutrients, did not differ significantly in any analysis and were consequently pooled.

RESULTS

The SLA increased significantly with the nitrogen fertilization gradient and ranged from 92 cm² per g in *Agropyron*, 82 in *Poa* and 118 in *Schizachyrium* in the plots with no fertilization to 169 in *Agropyron*, 105 in *Poa* and 165 in *Schizachyrium* in the highest fertilization plot where the species was present (Fig. 1). This represents an increase of 82% for *Agropyron* and 28% for *Poa*. *Schizachyrium* was absent from the three highest fertilization plots, but increased 40% in the plot with the most fertilizer where it occurred.

Species composition also changed along the nitrogen gradient with increasing dominance of *Agropyron* (Fig. 2a) with higher fertilization levels. Biomass (simple regression, $F = 8.7$, $n = 54$, $P = 0.005$, $R^2 = 0.143$) and LAI ($F = 56.7$, $n = 54$, $P < 0.001$, $R^2 = 0.519$) both significantly increased with increasing levels of nitrogen fertilization. However, the LAI of the vegetation changed 202% along the fertilization gradient whereas the aboveground biomass only increased by 57% (Fig. 2). One-third of this LAI increase resulted from the species shift (*Agropyron* replacing *Poa* and *Schizachyrium*) and two-thirds resulted from the change in SLA within *Agropyron*. Also noteworthy is that whereas the increase of SLA within species is gradual along the nitrogen fertilization gradient (Fig. 1), the biomass response is

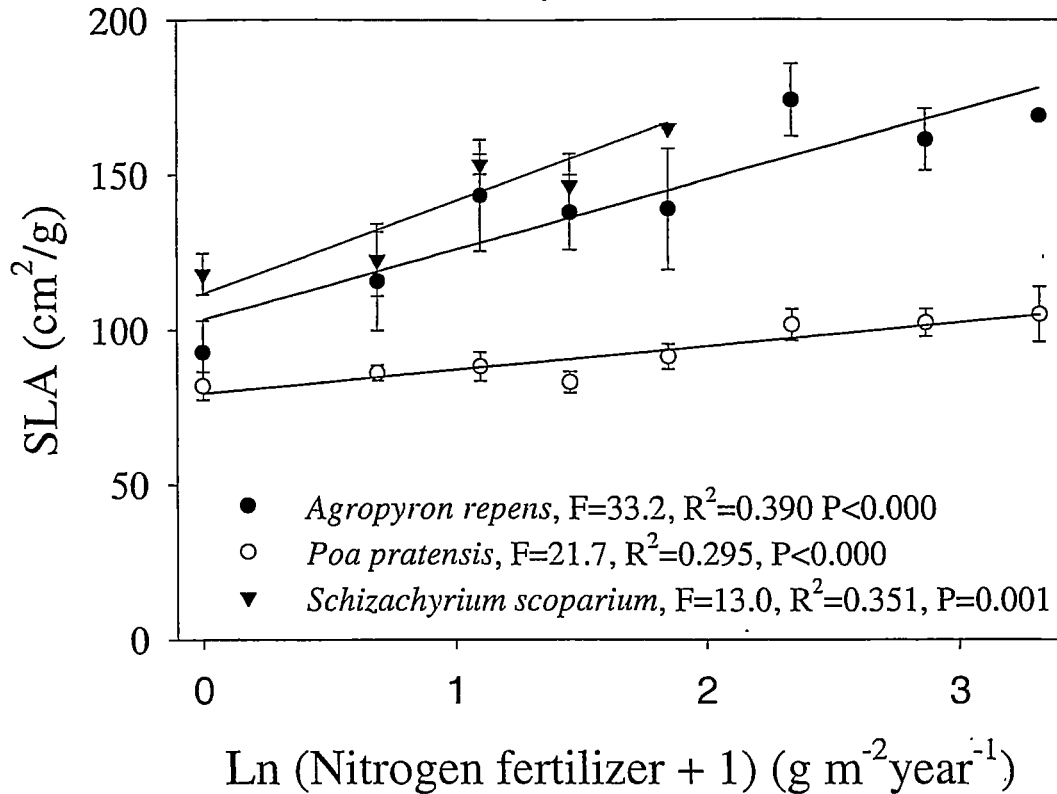


FIG. 1.—Specific leaf area of leaves of *Agropyron repens*, *Poa pratensis* and *Schizachyrium scoparium* along a nitrogen gradient. Note the nitrogen gradient is $\ln(x + 1)$ transformed. Data are means ± 1 SE in cm^2/g

not gradual because the decrease in biomass of *Schizachyrium* and *Poa* at the intermediate levels is only partly matched by the increase of *Agropyron* biomass (Fig. 2a).

DISCUSSION

Factors determining competition along a nitrogen gradient.—The tradeoff in biomass allocation between photosynthetic tissues and belowground tissues has been used to successfully model species replacement along nitrogen gradients (Tilman, 1988). However, a tradeoff in leaf longevity, with corresponding nitrogen allocation rates and growth ability can also explain species replacement along nitrogen gradients (Aerts, 1989; Berendse *et al.*, 1989; Aerts *et al.*, 1990). Note that leaf longevity, SLA, tissue nitrogen concentration and leaf gas exchange are correlated (Reich *et al.*, 1992; Shipley, 1995). Nitrogen fertilization influences the cost of nitrogen. Thus, at the lower end of the nitrogen gradient, species with a low SLA (and presumably long-lived leaves) dominate. Species which grow faster and have a higher leaf turn-over are at a disadvantage because they lose nitrogen when leaves die (Chapin and Kedrowski, 1983; Escudero *et al.*, 1992; Heckathorn and DeLucia, 1994). This dominance of species with low nitrogen levels in their tissue also results in nitrogen poor litter that retains all of its nitrogen during decomposition (Pastor *et al.*, 1987) and essentially all nitrogen gets incorporated into the soil organic matter (Wedin and Tilman, 1996). At higher nitrogen levels slower growing species are out competed because faster growing

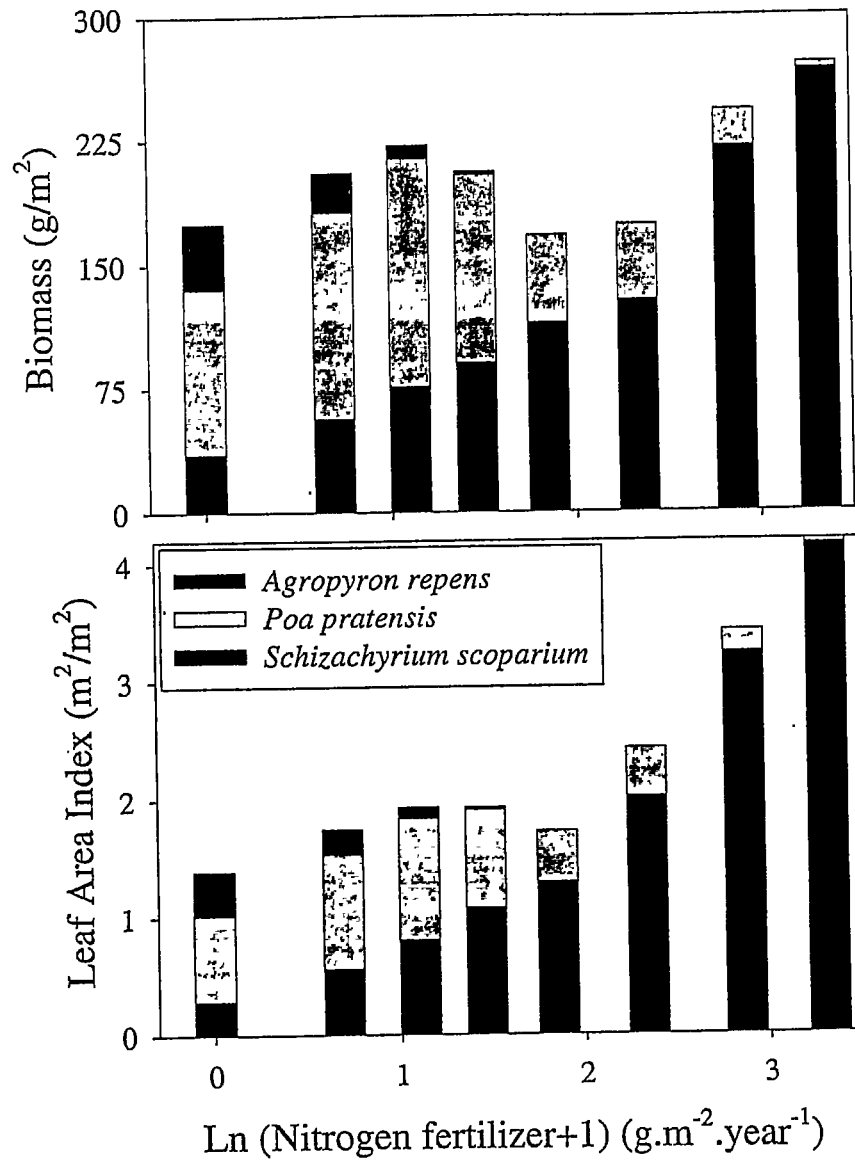


FIG. 2.—Total plot biomass, leaf area index and the contribution of *Agropyron repens*, *Poa pratensis* and *Schizachyrium scoparium*, along a nitrogen gradient. Other biomass ranges from 0.0 to 13.1 g/m² per treatment and averages 1.8% of the total life biomass per treatment (highest 4.6%). Note the nitrogen gradient is Ln(x + 1) transformed

species are able to place their leaves higher in the canopy, in a better light environment, and their nitrogen retranslocation cost is diminished by fertilization and increased nitrogen turnover from better quality litter (Wedin and Tilman, 1996). This is supported by Craine *et al.* (1999) who reported a leaf longevity of 13.1 ± 0.4 d for *Poa* vs. 7.5 ± 0.1 d for *Agropyron*. In addition, SLA often significantly correlates with leaf longevity (Dijkstra, 1989; Reich *et al.*, 1992). Note that these leaf longevity measurements are from unfertilized plots and that the increasing SLA of *Agropyron* along the fertilization gradient implies that the

leaf longevity of *Agropyron* might also be plastic and decrease even more. This is analogous to the competitive replacement of a shrub by a grass, induced by increasing nitrogen (Aerts, 1989; Aerts *et al.*, 1990; Berendse *et al.*, 1992). This tradeoff in leaf longevity, corresponding with SLA and growth rate, reinforces changes in biomass allocation (Tilman, 1988; Wedin and Tilman, 1993) in structuring the competitive replacement of species along fertility gradients.

Specific leaf area.—This study supports findings that SLA, in addition to aboveground / belowground biomass allocation (Tilman, 1988), is an important factor in determining the competitive ability of species along soil fertility gradients. It also supports Olf (1992) who argued that SLA and vertical stature were more important than biomass allocation patterns in explaining the position of species along a successional gradient. SLA is often reported as being the most significant factor in explaining differences in relative growth rate between species in laboratory studies (Poorter and Remkes, 1990; Garnier, 1992; Lambers and Poorter, 1992). The plasticity within *Agropyron* and the shift between *Poa* and *Agropyron* in this field study implies that SLA might influence part of the outcome of competition in ecosystems. This hypothesis in which the plasticity in SLA is an important component of the competitive ability of a species is also supported by a modeling study of Schieving and Poorter (1999).

The specific leaf areas reported are on the low end of the reported range for grasses (Garnier *et al.*, 1997; Van Arendonk *et al.*, 1997). However, most previous studies were conducted in the laboratory under optimal growing conditions (Poorter and Remkes, 1990; van der Werf *et al.*, 1993; Shipley, 1995; Van Arendonk *et al.*, 1997), which generally show higher SLA rates (Poorter and de Jong, 1999). Another previous study by Garnier *et al.* (1997) sampled in the spring in a Mediterranean climate, when there is ample rainfall. Our lower values might be due to the drier climate in Minnesota in the summer. Lastly, we also sampled whole plants and SLA decreases with age (Gunn *et al.*, 1999).

We found an increase in SLA with the nitrogen gradient, which is consistent with, but smaller than, laboratory data of *Poa* (Van Arendonk *et al.*, 1997) and other grass species (Poorter *et al.*, 1995). However, van der Werf *et al.* (1993) reported no changes in SLA between two fertilization levels of five grasses and Dijkstra (1989) found only marginal changes. A direct comparison of fertilization levels is not possible because these laboratory studies are performed with continuous fertilization, whereas our study used two fertilizations during the growing season.

Leaf area index.—LAI changed much more along the nitrogen gradient than plant biomass, which is consistent with the hypothesis that competition for light increases in the plots with more nitrogen (Tilman, 1988). Two-thirds of this disproportionate increase is due to plasticity within a species SLA, documenting its importance in changing the LAI within these experimental plots. This supports the hypothesis that plasticity of SLA as an important component of *Agropyron*'s ability to dominate at the higher nitrogen fertilization range and occur in the lowest nitrogen fertilization levels.

SLA is inversely correlated with leaf life-span (Reich, 1993). This relationship, combined with the substantially larger leaf area in the fertilized plots, indicates that there is a shift along the fertilization gradient towards more but thinner or less dense leaves with a higher turnover. Such a pattern supports the hypothesis that competition for light increases along this fertilization gradient, when the competition for soil nitrogen decreases. The increased competition for light might result in an intense light gradient within the canopy, increasing the competitive advantage of newly produced leaves high in the canopy, which results in decreased light availability lower in the canopy and a corresponding increase in leaf turnover. This is caused both by a plastic response of the species and species replacement. Also

noteworthy is the fact that *Agropyron*, which has a higher plasticity in SLA as compared to *Poa*, dominates in the highest fertilization plots. This supports the hypothesis that a tradeoff between the cost of newly produced leaves, the ability to retranslocate nitrogen from senescing leaves and the increased light levels that the newly formed leaves is an important factor in competition. Fertilization impacts the relative cost of nitrogen of producing new leaves and abscising old leaves. Note that we sampled in early July, the time that this C₃ grass dominated vegetation reaches its peak standing biomass. Species plasticity to develop new leaves and lose old leaves is evident but differences between species dominate. This is demonstrated by the difference in biomass between species along the fertilization gradients and the fact that *Schizachyrium*, which has a similar plasticity as compared to *Agropyron*, is competitive excluded at the highest fertilization levels. These results are consistent with laboratory studies (van der Werf *et al.*, 1993; Van Arendonk *et al.*, 1997) and field studies (Wedin and Tilman, 1993) of grasses grown at different nitrogen levels.

Acknowledgments.—Thanks to Peter Reich for the *Poa* leaf area measurements, Dave Tilman for the biomass measurements, Troy Mielke for help in the field, Charles Bristow for compiling the biomass data and Bryan Foster, Nick Haddad and Sarah Hobbie for comments. This work was supported by NSF grants DEB-9411972 and DEB-9629566.

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SUBMITTED 11 OCTOBER 1999

ACCEPTED 30 DECEMBER 1999