

THE ROLE OF NITROGEN IN GRASSLANDS: FROM ECOPHYSIOLOGY TO ECOSYSTEM AND COMPETITION TO HERBIVORY

Joseph M. Craine

Landcare Research, Dunedin, New Zealand.

ABSTRACT

Assembling a unified theory of the functioning of grasslands requires understanding how organisms work at multiple process levels, how they interact, and how physical factors such as soil type and climate affect ecosystem components. Nutrients in many grasslands are resources that link functioning at many scales and constrain the potential diversity of the function of ecosystems. Natural selection for growth in humid, low-nutrient environments has produced a plant strategy that includes low nutrient concentrations, low activity rates, and long lifespan for leaves and roots. These low-N traits serve as the basis for vertical integration of ecosystem processes as they are tightly linked to whole-plant morphology and reinforce low N availability. Thin, long-lived roots result in high root length density and low inter-root distance, which decrease the concentrations of N in soil solution. The low N concentrations of biomass strongly increase microbial N immobilization and are too low to support mammalian grazers. Consequently, humid grasslands burn instead of being grazed, removing a potential source of N return to the ecosystem. Despite these broad patterns, it is important to note that tight coupling occurs between plant traits and ecosystem processes. Many traits such as the N concentrations and activity rates of plant biomass are a consequence of plant effects on soil N availability. The causal nature of the relationships between plant traits such as N concentration and plant effects on soil N availability need to be determined. Many important differences in plants and ecosystems are independent of N status, e.g. the differences between monocots and dicots. Yet, arraying plants and ecosystems along an N gradient is a powerful explanatory and predictive tool for understanding ecosystem function.

1. INTRODUCTION

Assembling a unified theory of grasslands functioning requires understanding how organisms work at multiple process levels, how organisms interact, and how state factors such as soil type and climate affect the functioning and interaction of ecosystem components.

North American grassland ecologists during the early part of the 20th century focused on the importance of water in determining grassland form and function. For example, John Curtis (1959) differentiated Midwest prairies primarily based on soil moisture. John Weaver (1922) measured nitrate levels in soil early in his career, but most of his career focused on the importance of water in grasslands with little recognition of the importance of other nutrients. Weaver differentiated grasslands into shortgrass (xeric) and tallgrass (humid) and concentrated on comparing grasslands across precipitation gradients and following the effects of drought (Weaver 1968).

While water is a key resource determining the form and function of grasslands, nitrogen (N) is an equally key resource that links functioning at many scales. The availability of N constrains many ecological processes and simplifies the potential diversity of ecosystem functioning.

The recognition of the co-importance of nitrogen and water has been slow to develop (Hooper & Johnson 1999, Huntley & Walker 1982, Wedin 1995). An interesting chapter of the development in our understanding of nitrogen include the near simultaneous publication of some major ideas regarding how N-limited ecosystems function (referred to here as low-N ecosystems). The C-S-R hypothesis (Grime 1977), "The Mineral Nutrition of Wild Plants" (Chapin 1980), the application of R* theory of competition to terrestrial plants (Tilman 1980), and a synthesis volume that examined the functioning of tropical savannas (Huntley et al. 1982) all happened within a few short years of one another. More than 20 years were needed to develop and sort out the differences among these ideas and provide a integrated framework for N cycling, plant traits, competition and herbivory in low-N grasslands.

This paper does not attempt to be a comprehensive review on the functioning of low-N grasslands and or to rectify differences in the components of an integrated theory on the functioning of low-N ecosystems. Instead, I attempt to highlight my recent research in the field and bring in other relevant work at times. I focus on describing the suite of

integrated plant traits that are associated with low-N ecosystems, both at one low-N site (Cedar Creek Natural History Area in Minnesota, USA) and across grassland regions. Second, I examine the predictive ability of these traits in determining relative abundance of plant species across N supply gradients, focusing on the mechanisms of competition for nitrogen. Lastly, I discuss how N constrains herbivory and plant defenses.

2. PLANT TRAITS

We grew 33 species of grassland plants for five years on sandy soils with low N content at Cedar Creek Natural History Area (CCNHA), located in central Minnesota, USA (Craine et al. 2002). In this experiment, named E111, the species represented a range of native or naturalized species at CCNHA that included C₃ grasses, C₄ grasses, C₃ dicots (forbs), legumes, and two woody species. All species were perennial, except one annual forb. Biomass was harvested twice during the growing season. A range of measurements were made at the levels of organs, whole plants, and ecosystems.

One major axis of variation among species was associated with a suite of integrated traits that incorporated organ-level, whole-plant, and associated ecosystem traits, and separated those species that have low tissue N concentrations and are more productive under low N supplies from those that have higher tissue N concentrations and are less productive. We refer to these as low-N and high-N species.

Analyzing the traits of leaves and roots, low-N species had leaves that have low N concentrations, low rates of mass-based photosynthesis and respiration, low specific leaf area/high tissue density (Craine et al. 2002), and high longevity (Craine et al. 1999). This pattern was similar to that shown by Reich et al. (1997), who measured these factors for tree leaves across multiple regions. Belowground, low-N species had a suite of root traits that paralleled those of leaves. Low-N species had fine roots that have low N concentrations, low mass-based rates of respiration, high tissue density, and high longevity.

Plants that have low N concentrations in leaves had low N concentrations in roots. The evolutionary advantages of having low biomass N concentrations and high biomass longevity have yet to be resolved. Although, some have suggested that this minimizes N loss (Berendse, Elberse & Geerts 1992), at the whole plant level, low-N species lose as much N as high-N species (Craine et al. 2002). In E111, at the whole-plant level, N loss rates do not substantially differentiate low-N and high-N species. Instead, low N species were more productive because of higher C:N ratios. If N gain and loss rates can explain the differences among low-N and high-N species remains to be seen. Herbivory rates and/or physiological constraints may be necessary to explain the differences between low-N and high-N species.

At the whole-plant level, low-N species had greater standing biomass than high-N species. They also had high root:shoot ratios (R:S). Although it is commonly thought that high R:S is a result of high belowground allocation, we observed no differences in relative allocation rates belowground between low-N and high-N species (Craine et al. 2002). Instead, high R:S was a consequence of differential longevity of leaves and roots. Since the ratio of the longevity of roots to leaves (maximum = 3 y and 52 d) is greater in low-N species than high N species (minimum = 28 d and 28 d), biomass disproportionately accumulates belowground in low-N plants, leading to greater R:S.

Low-N and high-N species have similar relative belowground allocation rates, but differ in their patterns of allocation to reproduction and their growth form (Craine et al. 2001). Low-N species have shorter tiller distances, resulting in a tussock or bunch growth form. Also, the stems of low-N species function primarily to hold flowers up high and little leaf area is produced on the stems. Low-N species are more likely to be caespitose for a portion of the season with basal leaf area held at a high angle relative to the ground. If present on the stems of low-N species, leaves generally diminish in size with distance from the ground. In contrast, high-N species are more likely to be rhizomatous, are caulescent throughout the growing season, and have stems that not only hold flowers at height, but also serve to raise leaf area with leaves held parallel to the ground.

In E111, low-N species were also associated with lower rates of N mineralization and extractable N (Craine et al. 2002). These differences must have been due to feedbacks to the N cycle because all of the E111 plots began with the same initial condition. The high biomass production and low N concentrations increased microbial immobilization and lowered N availability (Wedin & Pastor 1993, Wedin & Tilman 1990). As such, low-N species that grow in low-N ecosystems have traits that reinforce low N availability by increasing microbial immobilization.

Although the above-described patterns were clear from a single experiment (Craine et al. 2002), further research shows that extreme caution must be used in determining which traits are primary traits of species, and which are consequences of other traits. Primacy and causality are not straightforward with highly correlated sets of traits. If two traits are correlated across a range of species, it is difficult to determine if one trait determines the other, or even if a third determines both of them. Because plants can affect the N supply and the N supply can affect the expression of traits, it is difficult to separate which traits are independent of N supply (or have the lowest plasticity) and which traits may be a consequence of the effects of plants on N supply.

For example, 10 species of CCNHA plants were grown from seed in containers in the field over 2.5 years in soils with low N content (Craine et al. in press, Craine et al. 2003). The species grown included two C₃ grasses, three C₄ grasses, three forbs, and two legumes. Replicates of each species were harvested at eight different times. During the first harvest, all species had relatively high tissue N concentrations. Yet, for the non-legumes over the remaining harvests, both tissue N concentrations and soil extractable N declined over time as plant biomass increased. Compared to high-N species, biomass accumulated at a greater rate in low-N species and tissue N concentrations and soil N availability dropped further. Although we consider the low tissue N concentrations of low-N species to be defining characters, it does not appear that the low tissue N concentrations are primary traits of the species. Low-N species do not necessarily have low-N concentrations. Instead they are a consequence of their effects on soil N availability.

The secondary nature of some traits extends beyond N concentrations and includes other traits such as leaf longevity. In another grassland experiment (BioCON) that was conducted on higher N soils and examined sooner after planting than E111, leaf longevity was measured on many of the same species that were in E111 (Craine & Reich 2001). There was no relationship between the longevity of leaves in BioCON and E111. For example, one species that had one of the longest leaf longevitys in E111 had the shortest in BioCON. There were similar relationships between leaf N concentrations and longevity among species between the two experiments. Yet, species differed in their location along this relationship, presumably due to the differences between the experiments and feedbacks to the N cycle.

In general, the N cycle must be directly incorporated into relationships among plant traits when describing the nature of suites of integrated traits. Both feedbacks to the N cycle and the dependency of trait expression on N availability require further attention for a more complete understanding of plant trait relationships. Although there are clear patterns of traits across species when grown long-term under low N supply, our understanding primacy and causality of traits is weak.

3. GLOBAL RELATIONSHIPS AMONG FUNCTIONAL TRAITS

In the experiments I described above which were at a single site, strong patterns among plant traits were quantified. These patterns paralleled what was seen by other researchers in other areas, e.g. Grime et al. (1997). Yet, there has been little work that compares functional traits across regions to understand the similarities and differences of plants of different regions.

To address regional relationships, we sampled 76 sites in four grassland regions of the world: the South Island of New Zealand, eastern South Africa, northern Australia, and the central grassland region of North America (a transect from Colorado to Illinois) (Craine & Lee 2003, Craine et al. submitted). We sampled multiple species at each site. For each species, we measured key leaf and root functional traits such as N concentrations, tissue density, $\delta^{15}\text{N}$, root thickness and leaf diameter. There were two main patterns among traits. Within each region we saw a gradient of plants from low-N to high-N species. Plants with low N concentrations in leaves had high leaf tissue density and low N concentrations in roots. In New Zealand, where we sampled the largest number of sites and consequently sampled the same species repeatedly, intra- and interspecific variation were similar for these relationships.

The differences observed in trait relationships among regions shows the necessity of extending one's results beyond a site or a region. The plants of the different regions primarily differed in their root traits. The plants of New Zealand and North America had thinner roots, lower root tissue density, and higher root N concentrations. The differences in root diameter were attributed to differences in nutrient limitation. Plants in Australian and South African sites are more likely to be P-limited and root diameter has been shown to be associated with dependence on mycorrhizae. New Zealand and North American plants had higher root N concentrations and lower tissue density than expected compared to northern Australia and South Africa. The New Zealand and North American sites experience freezing soils which may limit root longevity and the advantages for high root tissue density and/or low root N concentrations.

In general, there has been poor separation of fertility and stress or disturbance when analyzing root traits, which has caused some confusion to the relationship between leaf and root traits. For example, black spruce (*Picea mariana*) trees have leaves that live many years, but root longevity is less than a year. Though the ecosystems they are found in are N-limited, this does not necessarily mean that there is an inverse relationship between root and leaf longevity. The soils of these ecosystems are subjected to severe freezing, and the associated physical stresses are likely the cause of the low root longevity, not natural selection associated with N-limited ecosystems.

4. RELATIVE ABUNDANCE

The rationale for examining plant traits has long been to understand the distribution and abundance of species. Although we assumed that the low-N sets of traits were associated with superior performance and competitive ability under N-limitation, it was necessary to compare the ability of the N-axis of species to explain relative abundances of species in assemblages.

At CCNHA, based on a few key traits such as root and leaf tissue density and leaf angle, we created a quantitative continuous index of species from low-N to high-N species from measurements conducted on plants grown in the field in soils with low N content. Seventy-six species were measured in total, including annual and perennial species, C₃ grasses, C₄ grasses, forbs, and legumes. In one experiment (E001), plots were fertilized with N at rates ranging from 0 g N m⁻² to 80 g N m⁻² y⁻¹ for over 15 years with replicate blocks in four different fields from early to late successional old fields and intact savanna (Tilman 1987). The N-index of a plant was a strong predictor of its relative abundance across the N fertilization gradient (Craine et al. 2001). At low N fertilization rates, plots were dominated by low-N species. The proportion of high-N species increased as N fertilization rate increased. Similar patterns were seen across a disturbance and fertility interaction experiment, but additionally, high disturbance plots were dominated by high-N species. The only difference was that they were more likely to be annual high-N species, rather than perennial high-N species.

Across the four grassland regions, we also found that low-N species were associated with low-N ecosystems. We measured N supply over one year with *in situ* ion-exchange resins in bags in New Zealand. There was a strong positive relationship between the N concentrations of plants and N supply, with low-N plants growing in low N supply sites (Craine and Lee, unpublished). Across all four regions (New Zealand, South Africa, Australia, and North America), low-N plants were more depleted in ¹⁵N and had lower $\delta^{15}\text{N}$ (Craine et al. submitted). The greater enrichment of high-N sites must have been caused by processes that fractionate against ¹⁵N. Denitrification, nitrification, and acquisition of ammonium are associated with excess N in the soil and all lead to enrichment of ¹⁵N in plants. These relationships further strengthened the linkages between low-N/high-N plants and N supply.

5. COMPETITION FOR N

In experiment E111, plants were grown in low N content soils and after five years all the species we sampled were still present, though the high-N species had fewer individuals and less biomass overall. Competition is a very important determinant of the relative abundance of species. A few results observed in the above-mentioned experiments led to questions regarding the nature of competition for N. Although the phenomenon of plant competition for nutrients is well-studied, our understanding of nutrient uptake by plants has yet to be integrated into competition theory as fully as have light supply and acquisition (Tinker & Nye 2000).

The R* theory of competition states that the most competitive species for a limiting nutrient is that which can lower the availability of the limiting nutrient the most and be able to grow at that low availability (Tilman 1988). For nutrients in terrestrial ecosystems, nutrient availability has been interpreted as the concentration of nutrient in soil solution, analogous to work for planktonic algae grown in well-mixed solutions (Tilman 1976). Consequently, for terrestrial ecosystems, when the concentration of nutrient in soil solution is lowered below the minimum concentration required for growth and reproduction for a species, that species is supposed to be eliminated competitively.

A few lines of evidence in the CCNHA research were contrary to the R* theory. First, species that had lower R* (lower concentrations of nitrate and ammonium in soil solution) when grown in monoculture were not the competitively dominant species. *Solidago rigida*, a forb that is present in old fields at Cedar Creek but not more competitive than C₄ grasses (e.g. *Andropogon gerardii* and *Schizachyrium scoparium*) had lower soil solution concentrations (R*) than the C₄ grasses (Craine et al. 2003). Second, the minimum concentrations at which the C₄ grasses and *Solidago rigida* can take up nitrogen are an order of magnitude lower than those seen in the soils (Craine and Silim, unpublished). Plants should not be outcompeted necessarily because the concentrations of N in soil solution are too low. At the average concentrations seen in soils, competitively inferior plants should still be able to grow.

The key to resolving this discrepancy is that for nutrient-limited plants growing in the absence of competition, long-term nutrient uptake is constrained by nutrient supply (Leadley, Reynolds & Chapin 1997). Individual roots have to wait for nutrients to arrive at the root surface. Uptake is relatively insensitive to the concentrations of nutrients in soil solution, root surface area per unit root length, and the uptake kinetics of roots. Only producing additional root length in unexplored soils or increasing the mineralization of nutrients in explored soils increases nutrient uptake. When root systems of two plants are competing for nutrients, partitioning of the nutrient supply is most dependent on and proportional to the relative amounts of root length of the two individuals (Smethurst & Comerford 1993). Each

additional unit of root length for an individual acquires a greater proportion of the nutrient supply by preempting nutrients from arriving at the roots of its competitor. I refer to this as steady-state resource preemption.

The steady-state resource preemption theory differs fundamentally from the R^* theory of resource competition. The steady-state resource preemption theory focuses on the supply of the limiting nutrient in solution, while the R^* model of competition states that competitive superiority is dependent on the concentration of the nutrient. Since nutrient concentrations in soil solution are not necessarily correlated with nutrient supply and plants can acquire nutrients at concentrations much lower than average nutrient concentrations in the soil solution, the concentration of limiting nutrients in soil solution can not have the importance that R^* theory supposes.

For competition for N by plants, the key trait is root length density—the length of root per unit soil volume. Uptake by a plant is proportional to its fraction of the total root length density. Plants that can produce and maintain more root length should be better competitors for N. The traits that are associated with high root length density are the same that are seen in many of the low-N species. Low N concentrations allow plants to produce more root biomass per unit N. High root longevity allows root length that is produced to accumulate. Roots systems with thinner roots will have higher root length, all other things equal. Grasses on average have thinner roots than forbs (Craine et al. 2001, Grime et al. 1997), which serves as a simple explanation for why *Solidago rigida* is less competitive than the C_4 grasses and why grasslands in general are dominated by grasses and not forbs.

5. HERBIVORY

The consequences for the low-N suite of traits extends beyond production and competition to include higher trophic levels. Another characteristic of humid, low-N ecosystems is low mammalian herbivore abundance (East 1984). Sites with sustained nutrient input (floodplains) and dry sites have greater herbivore abundance than humid nutrient limited grasslands, even though production is still high. The low herbivore abundance results from an interplay between the N requirements of animals (and their microbial flora) and the N concentrations of plants.

All mammalian grazers rely on microbes to digest the non-soluble fractions of plants. The soluble fraction of plant biomass is released during chewing, but non-soluble fractions must be digested by microbes (Van Soest 1982). The microbial gut flora are under similar N constraints as those in the soil. If their food supply has an N concentration that is too low, available N is retained in microbial biomass (microbial immobilization). Only when the N concentrations are high enough are amino acids released (or not taken up), the equivalent of mineralization of N by soil microbes. Increasing the mean residence time of the food in the gut increases the amount of C that is respired or processed and increases the N concentration of the food, but there are constraints to the maximum mean residence time of food (Owen-Smith 1982).

When N concentrations are too low, microbial digestion slows while immobilization increases and less N becomes available to the animal. This minimum N requirement decreases with increasing body size, but is thought to be about 1% in large ruminants. If N concentrations are higher than this, amino acids and proteins are made available to the animal. If it is lower than 1%, the animal is faced with catabolizing N from muscle and/or recycling urea N to keep the microbial flora functioning. Although recycling urea helps to maintain a positive N balance, a large amount of N passes in fecal material and animals faced with a low N concentration diet have net N losses. Non-ruminants are able to selectively pass coarse material that has a high C:N, but the same constraints apply to minimum N concentrations of these animals.

The consequence of having a minimum N requirement is that in ecosystems where average N concentrations in biomass are less than 1%, herbivores can not be supported year-round, unless they preferentially select high quality forage. Ecosystems that have plant biomass that have N concentrations greater than 1% for part of the year, are often associated with seasonal movements into the region when quality is high. Lactation exerts extra N demands on females and generally even higher quality forage is required.

As a result of these N constraints, most low-N grasslands have low densities of mammalian herbivores. Humid grassland have lower average shoot N concentrations than xeric grasslands and can have low densities of grazers even though the quantity of food is high. Across precipitation gradients, N concentrations decrease with increasing precipitation. This has been observed in Africa, Australia, China, and North America (Bremner & de Wit 1983, Ellery, Scholes & Scholes 1995, Ting-Cheng 1993). Controlling for nutrient inputs and seasonal migrations, with increasing precipitation, animal abundance increases as a result of increasing quantity, but then decreases as the quality decreases. The one apparent exception to the quality rule comes from termites, whose abundance increases with increasing precipitation. They are most abundant in humid grasslands where mammalian grazer abundance is low. The reason the quality constraints do not apply to termites is that they have N_2 -fixing microbes in their gut flora. This enables termites to consume material that has lower N concentrations than 1% and still maintain their N balance.

The N concentrations of biomass determine herbivory rates, and set the potential for fire. A small fraction of production is eaten in low-N ecosystems. With sufficient ignition events, low-N ecosystems burn instead of being grazed. Some systems such as New Zealand grasslands are low-N ecosystems, have low herbivory, but low fire frequency. This results from some combination of broken topography, low lightning, and only recent human settlement which limit the ignition of biomass and the spread of fire.

6. DEFENCES

Even though herbivory rates are low in low-N ecosystems as a result of insufficient protein concentrations, plants still must be defended against herbivory. If the N concentrations of any part of the plant are greater than 1% at some point during the year (or termites are around), herbivory is likely.

Grasses have silica in their leaves which is thought to serve as a defense against herbivory. In contrast to forbs, very few grasses have secondary compounds. *Cymbopogon* and *Bothriochloa* species have phenolic compounds. A *Stipa* in the southwest United States has a narcotic. Unless endophytes are present, the large majority of grass species are not chemically defended. Grasses persist because they can outcompete forbs due to having thinner roots, but if grazing pressure is too high, forbs with their chemical and structural defenses dominate.

The defenses of dicots in N-limited ecosystems tend to be C-based. According to the resource availability hypothesis (Coley & Barone 1996), N is too expensive to use as a defense. N₂-fixers will use N-containing defenses in low-N ecosystems since N is cheaper for them, but N-containing defenses are not prevalent in non-fixers in N-limited ecosystems until N availability increases.

By extending economic models of shoot resource balance to include the relative value of C and N, depreciation, and amortization, we were able to show how the costs and benefits of defense types changed across an N-supply gradient (Craine et al. submitted). The key was calculating the relative value of N and C by quantifying their exchange rates at the whole plant or stand level.

We used a model of stand photosynthesis to calculate stand-level uptake of C across an N supply gradient. At low N supply, the ratio of C and N acquisition is high and declines with increasing N supply as stand photosynthesis saturates. As such the relative value of N declines with increasing N supply. With a quantitative value of N in terms of C, we calculated the net resource balance of shoots that used different defenses. At low N supply, N is too expensive in terms of C to be used for defense and plants that use C-based defenses have greater net resource acquisition. More as a result of the geography of the investigators that worked on defenses rather than any other reason, structural defenses had never been incorporated into resource availability theories. Although structural defenses like spines or the wireplant strategy of New Zealand shrubs (Bond, Lee & Craine submitted) rely on extra C for defense, these defenses are found more on high N supply sites, not low.

Our calculations showed that structural defenses require too much C for low-N leaves to photosynthesize and support the rest of the plant, especially since leaves are not completely protected. Since total C uptake increases at high N supply, structural defenses become more favored, both because C is abundant and the N lost from leaves costs less.

7. CONCLUSIONS

Nitrogen is a key element that links multiple components of ecosystem functioning, from the construction of plants to plant production to herbivore abundance. Although we are not exactly sure how or why, N limitation selects for plants with a suite of integrated traits such as low N concentrations and high longevity in leaves and roots and traits that reinforce low N availability. More cross-regional studies are necessary to understand important variation in grassland function and interaction between N and other factors such as climate or resource levels. Though more work is necessary, competition for N appears to require preemption of the N supply by maintaining root length dominance, not by lowering the concentrations of N in soil solutions. Lastly, the low N concentrations of plants constrain mammalian herbivory and plant defenses against it.

While a more complete review of the topic is necessary, a synthetic understanding of low-N grasslands is close at hand. Our current understanding still should go a long way to helping understand other processes such as invasions, restoration of ecosystems, and the consequences of N deposition.

8. REFERENCES

- Berendse, F, Elberse, WT and Geerts, RHME 1992. Competition and nitrogen loss from plants in grassland ecosystems. *Ecology* 73: 46-53.
- Bond, WJ, Lee, WG and Craine, JM submitted. Gondwana's evolutionary legacy: plants defended against large avian browsers. *Oikos*
- Breman, H and de Wit, CT 1983. Rangeland productivity and exploitation in the Sahel. *Science* 221: 1341-1347.
- Chapin, FS, III. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11: 233-260.
- Coley, PD and Barone, JA 1996. Herbivory and plant defenses in tropical forests. *Annual Reviews Inc*, P.O. Box 10139, 4139 El Camino Way, Palo Alto, California 94306, USA 1996.
- Craine, J, Bond, W, Lee, W, Reich, P and Ollinger, S submitted. The resource economics of chemical and structural defenses across nitrogen supply gradients. *Oikos*
- Craine, JM, Berin, DM, Reich, PB, Tilman, DG and Knops, JMH 1999. Measurement of leaf longevity of 14 species of grasses and forbs using a novel approach. *New Phytologist* 142: 475-481.
- Craine, JM, Froehle, J, Tilman, DG, Wedin, DA and Chapin, FS III 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos* 93: 274-285.
- Craine, JM and Lee, WG 2003. Leaf traits predict root traits across New Zealand grasslands. *Oecologia* in press:
- Craine, JM, Lee, WG, Bond, WJ, Williams, RJ and Johnson, LC submitted. Environmental constraints on a global relationship among leaf and root traits. *Ecosystems*
- Craine, JM and Reich, PB 2001. Elevated CO₂ and nitrogen supply alter leaf longevity of grassland species. *New Phytologist* 150: 397-493.
- Craine, JM, Tilman, DG, Wedin, DA, Reich, PB, Tjoelker, MJ and Knops, JMH 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Functional Ecology* 16: 563-574.
- Craine, JM, Wedin, DA, Chapin, FS, III and Reich, PB in press. Development of grassland root systems and their effects on ecosystem properties. *Plant and Soil*
- Craine, JM, Wedin, DA, F S Chapin, I and Reich, PB 2003. Relationship between the structure of root systems and resource use for 11 North American grassland plants. *Plant Ecology* 165: 85-100.
- Curtis, JT 1959. *The Vegetation of Wisconsin*. University of Wisconsin Press,
- East, R 1984. Rainfall, soil nutrient status and biomass of large African savanna mammals. *African Journal of Ecology* 22: 245-270.
- Ellery, WN, Scholes, RJ and Scholes, MC 1995. The distribution of sweetveld and sourveld in South Africa's grassland biome in relation to environmental factors. *African Journal of Range & Forage Science* 12: 38-45.
- Grime, JP 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.
- Grime, JP, Thompson, K, Hunt, R, Hodgson, JG, Cornelissen, JHC, Rorison, IH, Hendry, GAF, Ashenden, TW, Askew, AP, Band, SR, Booth, RE, Bossard, CC, Campbell, BD, Cooper, JEL, Davison, AW, Gupta, PL, Hall, W, Hand, DW, Hannah, MA, Hillier, SH, Hodgkinson, DJ, Jalili, A, Liu, Z, Mackey, JML, Matthews, N, Mowforth, MA, Neal, AM, Reader, RJ, Reiling, K, Ross-Fraser, W, Spencer, RE, Sutton, F, Tasker, DE, Thorpe, PC and Whitehouse, J 1997. Integrated screening validates primary axes of specialisation in plants. *Oikos* 79: 259-281.
- Hooper, DU and Johnson, L 1999. Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. *Biogeochemistry* 46: 247-293.
- Huntley, BJ and Walker, BH 1982. *Ecology of Tropical Savannas*. Springer-Verlag, Berlin.
- Leadley, PW, Reynolds, JF and Chapin, FS 1997. A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: Ecological implications. *Ecological Monographs* 67: 1-22.
- Owen-Smith, N 1982. Factors influencing the consumption of plant products by large herbivores. In: Huntley, BJ and Walker, BH (ed.). *The ecology of tropical savannas*. Springer-Verlag, Berlin.
- Reich, PB, Walters, MB and Ellsworth, DS 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America* 94: 13730-13734.

- Smethurst, PJ and Comerford, NB 1993. Simulating nutrient uptake by single or competing and contrasting root systems. *Soil Science Society of America Journal* 57: 1361-1367.
- Tilman, D 1976. Ecological competition between algae: Experimental confirmation of resource-based competition theory. *Science* 192: 463-465.
- Tilman, D 1980. Resources: a graphical-mechanistic approach to competition and predation. *American Naturalist* 116: 362-393.
- Tilman, D 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs* 57: 189-214.
- Tilman, D 1988. Plant strategies and the dynamics and function of plant communities. Princeton University Press, Princeton.
- Ting-Cheng, Z 1993. Grasslands of China. In: Coupland, RT (ed.). *Natural grasslands: eastern hemisphere and résumé*. Elsevier, Amsterdam.
- Tinker, PB and Nye, PH 2000. *Solute movement in the rhizosphere*. Oxford University Press, New York.
- Van Soest, PJ. 1982. *Nutritional Ecology of the Ruminant*. O & B Books, Corvallis, Oregon.
- Weaver, JE 1968. *Prairie Plants and their Environment: a fifty year study in the Midwest*. University of Nebraska Press, Lincoln, Nebraska, USA.
- Weaver, JE, Jean, FC and Crist, JW 1922. *Development and activities of roots of crop plants*. Carnegie Inst. Washington,
- Wedin, DA 1995. Species, nitrogen and grassland dynamics: the constraints of stuff. In: Jones, C and Lawton, JH (ed.). *Linking Species and Ecosystems*. Chapman and Hall, New York.
- Wedin, DA and Pastor, J 1993. Nitrogen mineralization dynamics in grass monocultures. *Oecologia* 96: 186-192.
- Wedin, DA and Tilman, D 1990. Species effects on nitrogen cycling: A test with perennial grasses. *Oecologia* 84: 433-441.