

Variation in soil moisture and N availability modulates carbon and water exchange in a California grassland experiment

Samuel B. St.Clair^{1,5}, Erika Sudderth², Marc Fischer³, Margaret Torn³, Stephanie Stuart², Rohit Salve³, Dennis Eggett⁴, David Ackerly^{2,3}

¹Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT 84602, USA

²Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

³Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

⁴Department of Statistics, Brigham Young University, Provo, UT 84602, USA

⁵Corresponding author, email: stclair@byu.edu, Tel: 801-422-5725, Fax: 801-422-0008

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Abstract

Variability in the magnitude and timing of precipitation is predicted to change under future climate scenarios. The primary objective of this study was to understand how variation in precipitation patterns consisting of soil moisture pulses mixed with intermittent dry down events influence ecosystem gas fluxes. We characterized the effects of precipitation amount and timing, N availability, and plant community composition on whole ecosystem and leaf gas exchange in a California annual grassland mesocosm study system that allowed precise control of soil moisture conditions. Ecosystem CO₂ and fluxes increased significantly with greater precipitation and were positively correlated with soil moisture. A repeated 10 day dry down period following 11 days of variable precipitation inputs strongly depressed net ecosystem CO₂ exchange (NEE) across a range of season precipitation totals, and plant community types. Ecosystem respiration (R_e), evapotranspiration (ET) and leaf level photosynthesis (A_{max}) showed greatest sensitivity to dry down periods in low precipitation plots. Nitrogen additions significantly increased NEE, R_e and A_{max}, particularly as water availability was increased. These results demonstrate that N availability and intermittent periods of soil moisture deficit (across a wide range of cumulative season precipitation totals) strongly modulate ecosystem gas exchange.

Introduction

Human activities are simultaneously altering global temperature, atmospheric CO₂ concentrations, nitrogen deposition rates and precipitation patterns (IPCC, 2007). These changes have the potential to drastically change plant function and ecosystem interactions at a global scale (Nemani *et al.* 2003). Precipitation patterns are a defining characteristic of earth's biomes (Gurevitch *et al.* 2006) and are a primary controller of ecosystem composition and function (Knapp and Smith, 2001). Soil moisture is an important integrator of terrestrial ecosystems responses to resource and temperature shifts associated with climate change (Weltzin *et al.* 2003). For example, the effects of elevated CO₂ and N deposition on plant productivity vary considerably depending on water availability (Poorter and Perez-Soba, 2001; Schimel *et al.* 1997). One of the major influences of warming on ecosystem function occurs through soil moisture loss with increased rates of evapotranspiration (Calanca *et al.* 2006; Harte *et al.* 1995).

Grasslands have been used as model systems to understand ecosystem responses to human alterations of global resource cycles and climate-related changes (Field *et al.* 1996). Grasslands are particularly responsive to variability in precipitation (Knapp and Smith, 2001). Several studies conducted in California annual grasslands have demonstrated neutral to positive effects on productivity to increases in annual precipitation (Dukes *et al.* 2005; Suttle *et al.* 2007; Harpole *et al.* 2007b). Far fewer studies have focused on California grassland ecosystem responses to conditions of water deficit because it is difficult to control ambient precipitation in field studies. However, recent climate models suggest that California and much of the subtropics, where grasslands are abundant, are more likely to experience increases in drought particularly

during the winter growing season when these grasslands are most active (IPCC, 2007; Hayhoe *et al.* 2004).

While cumulative, annual precipitation averages are an important determinant of grassland productivity, there is good evidence that variation in intra-seasonal precipitation patterns significantly influences the functional trajectory of grasslands. For example, the physiology and productivity of a perennial grassland was significantly decreased by increasing the magnitude and time interval between rainfall events, without changing annual precipitation totals (Knapp *et al.* 2002). Reduction in productivity was partially attributed to more frequent and prolonged periods of soil moisture deficit associated with increased variability in soil water content (Knapp *et al.* 2002). Understanding the influences of more variable weather patterns on grassland function is important as one of the characteristics of both recent and projected climate trends is more variable and extreme precipitation patterns (Sun *et al.* 2007; Groisman *et al.* 2005; Easterling *et al.* 2000), resulting in greater variation in soil moisture content and increases in the frequency and duration of soil moisture deficit (Harper *et al.* 2005).

Nitrogen is the element that most commonly limits biological productivity of temperate terrestrial ecosystems (Vitousek, 2004), including grasslands (Harpole *et al.* 2007a; LeBauer and Treseder, 2008). Nitrogen inputs into the global N cycle have doubled as a result of human activity and are predicted to continue rising (Galloway *et al.* 2004; Vitousek *et al.* 1997a). Ecological consequences of human alteration of the global N cycle include depletion of soil base cations, increases in stored terrestrial carbon and loss of species diversity (Fenn *et al.* 2003a; Vitousek *et al.* 1997b). The effects of N dynamics on ecosystem function are likely modulated by precipitation patterns. The size

and flux of soil N pools, as influenced by atmospheric inputs (Fenn *et al.* 2003b), microbial activity (Stark and Firestone, 1995), and losses are largely determined by precipitation events (Schimel *et al.* 1997). It is therefore critical to understand how ecosystem responses to N availability may be affected by the timing and intensity of precipitation events.

Gas exchange provides an integrated view of ecosystem water relations and carbon metabolism. Studies characterizing ecosystem gas flux responses to pulses of water have been conducted in annual (Harpole *et al.* 2007b) and semi-arid grasslands (Huxman *et al.* 2004; Patrick *et al.* 2007; Potts *et al.* 2006). However, a better understanding of how intermittent periods of water deficit influence ecosystem processes is lacking. We used a mesocosm experiment with automated watering and soil moisture sensing capacity in a climate controlled greenhouse which allowed dynamic control of soil moisture conditions which as mentioned above is extremely difficult to achieve in field studies. The primary objective of this study was to characterize how variation in precipitation amounts and timing, N availability and plant community composition influence CO₂ and H₂O fluxes in a mesic California annual grassland system. We tested the following hypotheses: 1) gas exchange rates increase concomitantly with precipitation; 2) intermittent periods of soil moisture deficit negatively affect grassland gas exchange rates, particularly when cumulative water status is low; 3) greater precipitation increases responsiveness to N; 4) Sensitivity of ecosystem gas exchange to variation in soil moisture and N availability varies with plant community composition.

Materials and Methods

Soil and seed collection

Soil was collected at the Hopland Research and Extension Center in Spring 2005. The site was prepared for soil collection by removing native vegetation. Soil developed on sandstone parent material (Haplustept), with A, B1 and B2 horizons that averaged 20 cm thick at the source site. Field bulk densities of 1.15, 1.4, and 1.6 g cm⁻³ for the A, B1 and B2 horizons, were determined. The soil was excavated by horizon, using a grader, and transported to the greenhouse facility in Richmond, California where the experiment was to be conducted. Each horizon's soil was then sieved to roughly 8 mm using a large screen. Seeds for all species (see below) were also collected at the Hopland Research and Extension Center in June of 2005 and 2006, in the vicinity of the soil collection site.

Plant growth conditions

Mesocosms, 57 cm in diameter and 66 cm tall, were constructed from thick wall PVC. A screen and fine sand were placed above a drainage hole to ensure adequate drainage. In the mesocosms, the native soil profile was reconstructed by packing each 20-cm horizon in its original position. To achieve the target bulk density for each horizon among mesocosms, we put the same mass of a given horizon soil into each mesocosm, and packed it to the correct volume. Twice we applied 10 mm of water to the soil surface of each mesocom to induce germination of the native seed bank which we removed upon germination so that we could control plant community composition. Following native

vegetation removal the soils were then allowed to air dry in the greenhouse for several weeks prior to initiation of the experiment.

The experiment was conducted in a climate-controlled greenhouse in Richmond, CA (37°54'50" N, 122°19'40" W). We used an unreplicated randomized complete block design with three treatment factors and five blocks. The treatments included three different plant communities, three water levels and two nitrogen levels. The first vegetation type was a mixed grass-forb community consisting of five grasses and two forbs sown at the following density (seeds m⁻²): *Avena barbata* (1500), *Bromus hordeaceus* (1500), *Briza maxima* (1000) *Hordeum murinum* (1000) *Aegilops triuncialis* (500) *Erodium botrys* (250) and *Amsinckia douglasiana* (250). *Avena* and *Erodium*, representing a dominant grass and forb, were grown in monoculture communities at densities of 4000 and 2000 seeds/m⁻² respectively. Sowing densities and species proportions were based on composition and density studies conducted in California annual grasslands (Pitt and Heady, 1978; Corbin *et al.* 2007).

The experiment was conducted for two growing seasons (2005-2006, 2006-2007) beginning with wet up in late November and ending with plant senescence and dry down in mid May. During the 2005 wet up period each mesocosm received a total of 45 mm of water over an 11 day period prior to imposing the precipitation treatment so that seed germination would be uniform. In the 2006 wet up period each mesocosm received 27 mm of water over a 7 day period. During the 2005-2006 season, precipitation treatments (cumulative season totals) included dry (315 mm year⁻¹), ambient (675 mm year⁻¹), and wet (1245 mm year⁻¹) conditions which are representative of the precipitation average and dry and wet yearly extremes as monitored at Hopland Research and Extension Center

from 1989-2005. During the 2006-2007 season the three precipitation treatments were adjusted slightly to 297 mm year⁻¹, 657 mm year⁻¹, and 987 mm year⁻¹.

The pattern of the precipitation treatments were designed based on an analysis of climate data from the Hopland station. We found that variation in total rainfall among years was primarily due to the number of rainy days, the intervals between rain events, and the duration of the season, whereas the average precipitation per rainy day did not vary between dry and wet years. According to this pattern each one day watering event was held constant at 15mm. Cumulative seasonal precipitation differences were the result of differences in the number of 15 mm precipitation events (high precipitation=10 (2005-06) or 8 (2006-07), ambient=6, low=3) applied within an 11 day watering period during a 21 day repeating water cycle. At the end of the 11 day watering period each of the three precipitation treatments experienced 10 days without water. The total number of 21 day watering cycles during the season also varied between the watering treatments (high=8, ambient=7, low=6) (Figure 1). The precipitation treatments were applied using an automated watering system designed to uniformly apply precise amounts of water across the soil surface during each irrigation event. Computer-controlled electronic relays operated programmable water pumps and a series of solenoids to precisely fill the water reservoirs associated with each mesocosm. Irrigation tubing connected the water reservoirs to two concentric rings of drip irrigation tubing on the soil surface of each mesocosm, through which water was slowly released.

In May of 2006 the above ground plant biomass and seeds were harvested from the mesocosms. In the summer of 2006 the mesocosms were re-seeded as explained above and 100 grams of the homogenized plant biomass without seeds (within each of the

three vegetation types) was added on as litter. 100 grams represented about 35% of the total above ground biomass produced in each of the vegetation types during the 2005-2006 growing season. Nitrogen was added in the form of ammonium nitrate (dissolved in water) and applied at a rate of 20 kg N ha⁻¹, twice during the spring of 2007 (at the beginning of water cycles 4 and 6) for a total of 40 kg N ha⁻¹. Leaf and canopy gas exchange, canopy height, leaf area index (LAI) and soil moisture were measured at the end of the 11 day wet period (wet max) and 7 days into the dry period during each water cycle during the 2006-2007 season.

Soil moisture in the mesocosms was monitored with horizontally installed 0.30 m long TDR probes, calibrated to provide the relationship between the dielectric constant (measured by the TDR probe) and the corresponding volumetric soil moisture content (SMC). A dielectric-SMC relationship was determined separately for each of the three soil horizons. During the first year of the experiment the TDR probes were located at two depths [0.10 m (center of the A horizon) and 0.50 m (center of the B₁ horizon)]. Before the start of the second year, an additional TDR probe was installed to monitor SMC content at 0.25 m depth (center of the B₂ horizon). Environmental sensors and a data logging system were used to measure and record air temperature, humidity and light intensity in 15 minute intervals. During the 2007 growing season mean temperature in the greenhouse ranged from 10-19°C with an average of 15 ± 2.4°C. Relative humidity ranged from 48-88% with an average of 70 ± 8.7%. Photosynthetic photon fluence rate (PPFR) varied throughout the day with a maximum of 1800 μmol photons m⁻² s⁻¹ and an average of 701 ± 322 μmol photons m⁻² s⁻¹.

Whole Ecosystem gas exchange measurement

A multi-segment flux chamber and infrared CO₂ and H₂O analyzer (Licor 7500) was used to measure ecosystem fluxes of CO₂ and water vapor (Patrick *et al.* 2007). The chamber was designed to fit directly on the top of the mesocosms. Positive seating to the mesocosm was accomplished with a bottom plate that included side walls to fit just inside and outside the mesocosm, with a foam gasket. The IRGA and a mixing fan were mounted to the chamber cover. The chamber height was increased by adding a second segment as the plants grew. Each chamber segment was 54 cm tall, and was comprised of circular top and bottom plates joined by eight rods. High transparency (> 95% for photosynthetically active radiation) 50 micron thick fluorocarbon plastic film (Dupont FEP) was used to cover the sides and top of the chamber. Using a flexible plastic film minimized soil-atmosphere pressure gradients that could dampen flux rates (Saleska *et al.* 1999). The importance of any small leaks in the chamber was estimated by introducing a gradient of 400 ppm CO₂ between the chamber and room air and measuring the loss of CO₂ from the chamber when the chamber was placed on a flat surface containing no soil or vegetation; the results were insignificant compared to typical ecosystem fluxes (i.e., < 0.1 (μmol CO₂ m⁻² s⁻¹)).

Ecosystem fluxes were determined by placing the chamber on a mesocosm and measuring the change in CO₂ and H₂O vapor concentrations over a 40-60 second interval. Whole ecosystem respiration was measured by placing a dark cloth over the chamber and repeating the measurement in the dark. Change in concentration with time, dC/dt (mmol CO₂ cm⁻³ s⁻¹), was obtained by fitting a linear regression of concentration on time after

removing the first 10 seconds of the measurement to allow for the instrument to settle and air to mix in the chamber. Typically, the linear fit captured between 80-95% of the variance in concentration, allowing dC/dt to be determined to within a few percent. Approximating the chamber as a right cylinder, the CO₂ flux, F ($\mu\text{mol C m}^{-2} \text{s}^{-1}$), was determined from the change in concentration as: $F = h dC/dt$, where h (cm) is the height from the top of the chamber to the soil surface. In addition to the height of the chamber segments (54 or 108 cm), we included the distance to the soil surface from the bottom of the chamber which was measured periodically for each mesocosm.

Leaf gas exchange

Leaf gas exchange values were based on a measurement taken on the youngest fully expanded leaf of *Avena barbata* plants in the mixed and grass monoculture communities and *Erodium botrys* plants in the Erodium monoculture community. Leaf gas exchange was measured using the LI-COR 6400 gas exchange system (LI-COR, Lincoln, NE, USA). Gas exchange was measured at a photosynthetic photon flux density (PPFD) of 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (generated by the LI-COR 6400-40 LED light source) at ambient temperature and humidity. Baseline leaf and reference chamber CO₂ concentrations of 375- $\mu\text{mol mol}^{-1}$ were achieved using a LI-COR 6400 CO₂ mixer. Measurements were initiated by sealing the leaf in the chamber. When CO₂ concentrations in the leaf chamber reached a steady state (60-90 seconds), leaf gas exchange values were logged.

Measures of growth and canopy structure

From water cycle 3-8 average canopy height across individuals was estimated using a measuring stick. Leaf area index (LAI) was measured 5 cm above soil level using a ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, WA, USA).

Statistical Analysis

Statistical analyses focused on the 2006-2007 growing season. Repeated measures analysis of variance (ANOVA) was used to test the main and interactive effects of treatment conditions on gas flux rates, canopy height, leaf area index (LAI) and soil moisture (10 cm depth) using time as the ‘within’ factor (Gumpertz and Brownie, 1993). The first ANOVA model incorporated plant community composition (Veg), season cumulative precipitation (Ppt), and measured differences during the wet and dry periods (Dry) within the 21 day water cycle as independent fixed effects over water cycles 2-6. Block was included in the model as a random effect. The second statistical model was similar to the first except that we analyzed data from cycles 4-6 (following the N applications) allowing us to include the effects of N (Fert) as an additional independent variable. For NEE, light intensity (PAR) was included in both models as a covariate. Pearson correlation and a stepwise multiple regression model were used to characterize relationships among dependent variables. Statistical significance was defined as $\alpha \leq 0.05$. Dependent variables were tested for normality and homogeneity of variance using Shapiro-Wilk W statistics to determine the goodness of fit of data in normal quantile plots. A Box-Cox transformation was applied to the R_e and ET data to satisfy the assumptions of normality. All other data were found to be normally distributed.

Statistical analysis was performed using SAS (Version 9.1) and JMP (Version 7.0) statistical software (SAS institute, Cary, NC, USA).

Results

Soil moisture

Trends in soil moisture did not differ significantly among the three plant community types (Table 1, Veg). Average soil moisture content at 10 cm depth was 37% and 57% higher in ambient and high precipitation plots compared to low precipitation plots (Figure 2). Soil moisture content changes over the course of the season varied among the precipitation treatments (Table 1, Ppt x Time) with soil moisture gradually declining through cycle 6 in the low precipitation plots while being maintained in ambient and high precipitation plots (Figure 2). The soil moisture content consistently peaked at the end of each watering period and then decreased 30-60% by the end of each dry down period resulting in a strongly significant soil moisture dry down effect (Table 1, Dry). Nitrogen addition significantly influenced soil moisture content (Table 2, Fert). A pattern emerged in which N amendments promoted greater soil drying in the mixed plant community plots than the monoculture communities (Table 2, Fert x Veg) (Figure 2).

Ecosystem CO₂ and H₂O exchange

Ambient and high precipitation plots had season-integrated net ecosystem CO₂ exchange (NEE), dark respiration (R_e), and evapotranspiration (ET) rates that were significantly greater than the low precipitation plots (Table 1, Ppt). However, the differences averaged over cycles 2-6 were modest (+10-17.1%). As the growing season progressed, the low precipitation plots showed decreasing rates of NEE and ET relative to the ambient and high precipitation treatments (Table 1, Ppt x Time) (Figures 3 & 5). The dry down periods decreased NEE by 18% on average but did not significantly affect

R_e or ET (Table 1, Dry) (Figures 3-5). The significant Ppt x Dry interaction effect on R_e over cycle 2-6 (Table 1) appears to be a function of low precipitation plots showing greater sensitivity to dry down events than ambient or high precipitation plots (Figures 4). A similar significant effect is seen for NEE and ET during cycles 4-6 (Table 2) (Figures 3 & 5).

Nitrogen addition significantly increased NEE and R_e (Table 2, Fert), an effect that increased with greater water availability (Table 2 Ppt x Fert) (Figures 3-4). *Erodium* monoculture plots had significantly lower NEE than either of the grass dominated communities, but there were no significant differences in R_e and ET among the three plant community types (Table 1, Veg). Net ecosystem CO₂ exchange remained relatively stable over time, while R_e and ET increased as the season progressed (Table 1, Time) (Figures 3-5). The majority of significant interaction terms in the ANOVA models were changes in the main effects over time (treatment x time interactions) (Table 1).

Net ecosystem CO₂ exchange was most strongly correlated with leaf photosynthesis, canopy height, PAR and soil moisture content at 25 cm (Table 3). Those four factors explained 38% of the variation in NEE data in the multiple regression model. Whole ecosystem respiration was most strongly correlated with canopy height and leaf area index (Table 3) with canopy height, LAI, PAR and soil moisture content (25 cm) explaining 31% of the variation in the multiple regression model. Ecosystem evapotranspiration was most strongly correlated with canopy height and PAR with those two factors explaining 39% of the variation in the multiple regression analysis.

Leaf photosynthesis

The main effects of precipitation and dry down on leaf carbon fixation were statistically significant (Table 1, Ppt. and Dry). The sensitivity of leaf photosynthesis to dry down events was greatest in low precipitation plots (Table 1, Ppt x Dry). *Erodium* had higher rates of photosynthesis than *Avena* growing in mixed or monoculture communities (Table 1, Veg) (Figure 6). The effects of dry down and vegetation on leaf gas exchange did change significantly over time (Table 1). N addition significantly stimulated leaf carbon fixation (Table 2) but did not interact significantly with any of the other treatment factors. Leaf photosynthesis was most strongly correlated with soil moisture content (Table 3).

Canopy height and leaf area index

The vegetation in the ambient and high precipitation plots tended to be taller and had greater LAI than in the low precipitation plots (Table 1, Ppt). Height stimulation in response to N addition increased with greater water availability (Table 2, Ppt x Fert). The mixed and monoculture grass plots had LAI values that were 14% greater than the monoculture forb plots (Table 1, Veg). Height and LAI increased over time as expected (Table 2, Time).

Discussion

The objective of this study was to characterize the main and interactive effects of precipitation amounts and timing, N availability and plant community composition on California annual grassland gas exchange and productivity. The results support the first hypothesis that gas exchange rates and plant growth increase concomitantly with water availability. However, increases in CO₂ and H₂O fluxes in response to increased water availability were modest and soil moisture never explained more than 20% of the variation in the gas exchange data. The second hypothesis that intermittent periods of water deficit impair flux rates was largely supported by the data as the dry down events significantly decreased ecosystem and leaf gas fluxes particularly under lower cumulative precipitation conditions. Greater water availability generally increased NEE, R_e and canopy height sensitivity to nitrogen as outlined in the third hypothesis. The results generally did not support the fourth hypothesis as three plant communities tended to respond similarly to precipitation and N treatments.

In semi-arid grasslands, water pulses can strongly affect whole ecosystem and leaf level gas exchange (Huxman *et al.* 2004), particularly following extended periods of water deficit (Potts *et al.* 2006). Cumulative differences in water status during wet seasons have also been shown to significantly influence ecosystem gas exchange. Strong increases (50-100%) in NEE were observed in both Sonoran and Chihuahan desert ecosystem by increasing water inputs by 25-50% during the monsoon season (Patrick *et al.* 2007; Potts *et al.* 2006). Harpole *et al.* (2007) showed similar increases in NEE, R_e and ET at the end of the growing season in response to 30% increases in water inputs over the growing season. Our season integrated NEE, R_e and ET responses to increased

water availability were much more modest (10-17%) compared to these studies. What is evident however is that the precipitation effect became more pronounced as the season progressed (Table 1, Ppt x Time) (Figure 3). For example, by cycle 6 NEE and R_e in ambient and high precipitation plots were ~35% greater than in low precipitation plots which was much greater than the season integrated average. Our data suggest that for more mesic annual grassland communities in Mediterranean climates, differences in cumulative season precipitation have their strongest influence on ecosystem fluxes later in the season as plant biomass increases (March-May). The significant time effect on ecosystem gas fluxes that we observed in this study highlights the dynamic shifts in flux rates that occur over the growing season.

Late season shifts in ecosystem gas flux sensitivity to precipitation treatments are likely driven by both changes in soil moisture status and biomass accumulation. For example, rising vapor pressure deficit from winter to spring and increasing plant biomass accumulation will tend to increase water losses through evapotranspiration. The significant Ppt x Time interaction for soil moisture (10 cm) (Table 1) indicates that low precipitation plots lost soil moisture as the season progressed, while soil moisture was maintained in higher moisture plots (Figure 2). Greater soil moisture may also increase ecosystem flux rates by stimulating plant growth. Canopy height which increased concomitantly with greater water availability in this study (Figure 7), did explain a significant amount of the variation in ecosystem gas fluxes (Table 3).

Flux rates in our more mesic study system were much greater than seen in more sparsely vegetated desert ecosystems where water deficit is more extreme (Patrick *et al.* 2007; Potts *et al.* 2006). These higher flux rates are partially a function of more

favorable water relations which increases physiological function and tends to produce denser grass communities (Pitt and Heady, 1978). In this study plant canopy height, leaf area index (LAI) and plant biomass and abundance (data not presented) increased significantly in response to greater water availability. Greater plant community biomass would tend to increase ecosystem gas exchange capacity. However, increases in above ground biomass density in response to the alleviation of below ground resource constraints (e.g. water and N) will at a certain point result in competition for light, which can constrain whole canopy photosynthesis (Lane *et al.* 2000). In contrast, competition for light is rare in arid ecosystems with low net primary productivity (Kicklighter *et al.* 1999). This may partially explain why whole ecosystem flux rates only increased modestly in response to greater water availability in our system compared to more arid desert ecosystems.

We anticipated that higher precipitation plots would be less sensitive to the dry down periods and that sensitivity would increase later in the season as biomass increased. Among the most intriguing results of this study is that the dry down period within each water cycle consistently depressed NEE independent of cumulative precipitation status, and plant community type (Table 1, Figure 3). Interestingly, R_e and ET showed much less sensitivity to these dry down periods (Table 1, Figures 4-5). Changes in soil moisture (10 cm) were consistent with the NEE response in that the dry down effect was significant but it did not interact significantly with precipitation treatment, or plant community type (Table 1). During the dry down period ambient and high precipitation plots consistently had soil moisture content in the 20-30% range while low precipitation plots were in the 10-20% range (Figure 2). The observed decreases in flux rates in the

higher precipitation plots in response to the dry down period suggest that the soil moisture optimum for NEE is greater than 20% in our study system.

Late season carbon fluxes were negatively affected by N amendments in a California annual grassland study (Harpole *et al.* 2007b). In contrast, we found that N addition stimulated carbon fluxes and that the response increased with greater water availability. (Table 1). This result suggests that soil moisture content in low precipitation plots may limit N transport to roots and/or water is a more limiting resource than N. Nitrogen addition significantly stimulated both height growth, and above ground biomass (data not presented), suggesting that increases in carbon fluxes in response to N could have partially been the result of growth stimulation. Leaf level photosynthesis was strongly upregulated in response to N indicating that increases in NEE may also have been related to higher rates of photosynthesis.

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| Source of variance | df | NEE | R _e | ET | A _{max} | Canopy Ht | LAI | Soil moisture (10 cm) |
|--------------------|----|---------|----------------|----------|------------------|-----------|----------|-----------------------|
| Ppt | 2 | 16.13** | 38.91*** | 0.91 | 5.44* | 22.86*** | 4.75* | 12.65** |
| Dry | 1 | 16.90* | 0.92 | 0.27 | 7.28* | 6.43 | 7.32* | 178.66*** |
| Veg | 2 | 6.12* | 1.44 | 4.21 | 34.13*** | 136.30*** | 5.33* | 1.33 |
| Time | 4 | 3.55 | 20.99*** | 10.97*** | 4.40* | 112.41*** | 85.04*** | 24.21*** |
| Ppt x Dry | 2 | 1.73 | 5.85* | 2.57 | 9.38** | 5.50* | 0.77 | 0.84 |
| Ppt x Veg | 4 | 0.42 | 1.69 | 0.77 | 0.63 | 1.44 | 0.96 | 1.58 |
| Ppt x Time | 8 | 3.02* | 1.07 | 3.70*** | 0.52 | 4.73*** | 0.87 | 10.17*** |
| Dry x Veg | 2 | 0.75 | 0.02 | 0.54 | 6.41* | 1.06 | 2.12 | 0.62 |
| Dry x Time | 4 | 3.66* | 3.02* | 0.52 | 6.70** | 7.65* | 31.51** | 2.99* |
| Veg x Time | 8 | 7.07*** | 12.72*** | 0.77 | 4.91*** | 5.23*** | 5.78*** | 4.77*** |

Table 1. F-values from repeated measures ANOVA model testing the main effects and two-way interactions on whole ecosystem and level level gas exchange, canopy growth and structure and soil moisture content over time. The model includes data from cycles 2-6 but excludes the N addition plots. Ppt = cumulative precipitation treatment, Dry = difference in measured response seven days into the dry period relative to the soil moisture maximum during the watering period, Veg = difference explained by the three unique vegetation communities. Significance designated as *P ≤ 0.05, ** P ≤ 0.01 *** P ≤ 0.001.

| Source of variance | df | NEE | R _e | ET | A _{max} | Canopy Ht | LAI | Soil moisture (10 cm) |
|--------------------|----|----------|----------------|--------|------------------|-----------|----------|-----------------------|
| Ppt | 2 | 64.71*** | 86.58*** | 3.22 | 11.73** | 85.46*** | 3.10 | 114.21*** |
| Dry | 1 | 86.80*** | 2.97 | 1.07 | 58.58*** | 18.99* | 30.19** | 486.49*** |
| Fert | 1 | 34.63*** | 82.73** | 0.27 | 54.30*** | 11.10* | 4.99 | 8.34* |
| Veg | 2 | 0.52 | 8.61** | 5.78* | 149.17** | 26.17*** | 9.04** | 0.09 |
| Time | 2 | 3.22 | 11.53*** | 6.81* | 3.68 | 411.94*** | 14.41** | 23.84*** |
| Ppt x Dry | 2 | 15.23*** | 8.25*** | 6.66** | 29.32*** | 4.04* | 0.35 | 0.22 |
| Ppt x Fert | 2 | 11.59*** | 7.76*** | 0.40 | 0.47 | 21.99*** | 1.42 | 0.59 |
| Ppt x Veg | 4 | 1.69 | 5.42*** | 1.67 | 1.29 | 4.33 | 2.3 | 5.13*** |
| Ppt x Time | 4 | 8.12*** | 4.77*** | 1.42 | 1.93 | 14.21*** | 0.54 | 1.70 |
| Dry x Fert | 1 | 0.90 | 0.96 | 0.60 | 3.57 | 1.24 | 2.50 | 0.79 |
| Dry x Veg | 4 | 3.74* | 0.37 | 1.56 | 7.35*** | 0.82 | 2.20 | 0.25 |
| Dry x Time | 2 | 27.50*** | 30.81*** | 1.46 | 7.91*** | 27.08*** | 63.69*** | 0.69 |
| Fert x Veg | 2 | 3.20* | 0.06 | 1.00 | 1.77 | 0.30 | 2.33 | 4.34* |
| Fert x Time | 2 | 6.17** | 7.23*** | 0.18 | 1.94 | 3.60* | 1.51 | 0.91 |
| Veg x Time | 4 | 3.07* | 15.69*** | 0.19 | 38.49*** | 1.94 | 5.43*** | 2.79* |

Table 2. F-values from repeated measures ANOVA model testing the main effects and two-way interactions on whole ecosystem and level level gas exchange, canopy growth and structure and soil moisture content over time. The model includes data from cycles 4-6 which includes the N treatment plots (Fert). Significance designated as * $P \leq 0.05$, ** $P \leq 0.01$ *** $P \leq 0.001$.

| | NEE | R _e | ET | A _{max} |
|---------------------|---------|----------------|---------|------------------|
| Leaf photosynthesis | 0.33*** | 0.20*** | 0.01 | |
| Leaf transpiration | 0.15*** | 0.04 | 0.10*** | 0.36*** |
| Canopy height | 0.29*** | 0.42*** | 0.50*** | 0.14** |
| LAI | 0.13** | 0.38*** | 0.20*** | 0.13** |
| PAR | 0.35*** | 0.28*** | 0.57*** | 0.12*** |
| WC 10cm | 0.25*** | 0.13*** | 0.08** | 0.36*** |
| WC 25 cm | 0.33** | 0.19*** | 0.13*** | 0.42*** |
| WC 50 cm | 0.20*** | 0.13*** | 0.08* | 0.34*** |
| Soil Tm 3 cm | 0.06 | 0.44*** | 0.39** | 0.27* |
| Soil Tm 10 cm | 0.02 | 0.44*** | 0.34** | 0.29* |
| Soil Tm 25 cm | 0.03 | 0.41*** | 0.35** | 0.42*** |

Table 3. Correlation coefficients indicating the relationship between dependent variables in the experiment. Correlative relationships are based on data collected during cycles 4-6 during the 2007 season. Significance designated as *P ≤ 0.05, ** P ≤ 0.01 *** P ≤ 0.001.

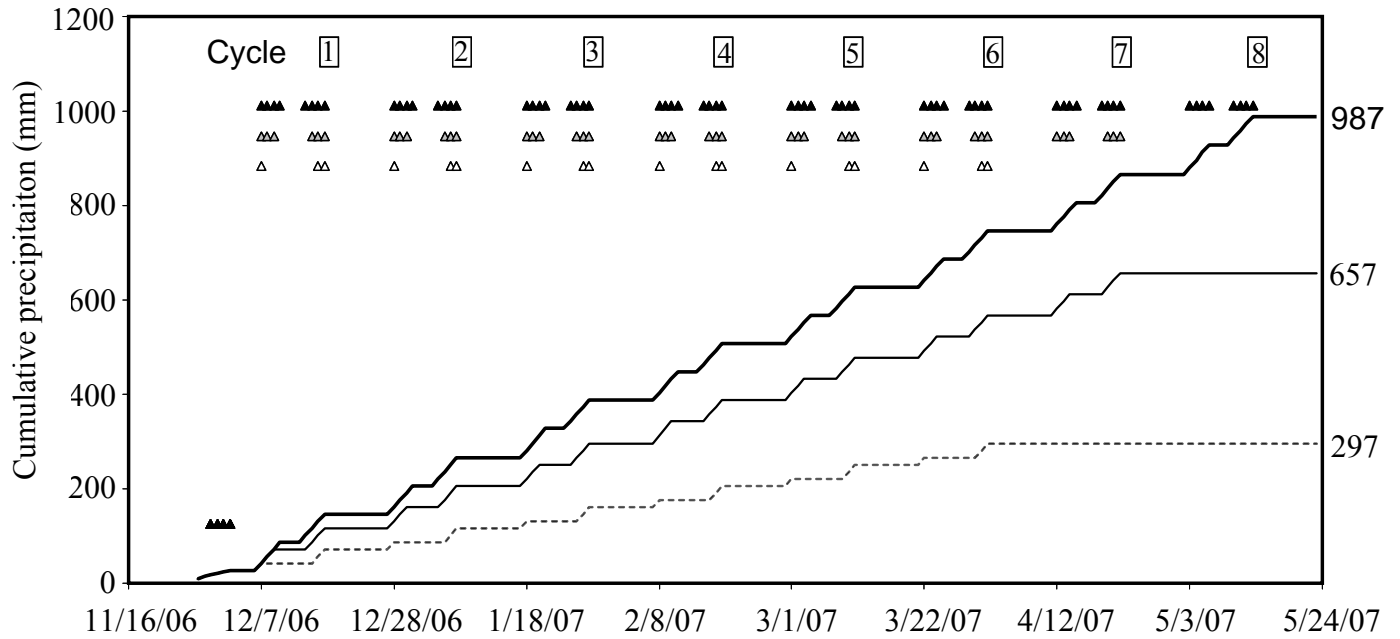


Figure 1. 2006-2007 precipitation regime. Within the 11 day watering period at the beginning of each of the 21 day water cycles the high ambient and low precipitation plots received eight, six and three 15 mm precipitation events respectively (each triangle symbol at the top of the graph represents a 15mm irrigation event). Each water treatment then experienced a uniform dry down period of 10 days at the end of each 11 day watering period within each water cycle. Cumulative season precipitation totals for each of the three treatments were 987mm, 657mm and 297mm, respectively.

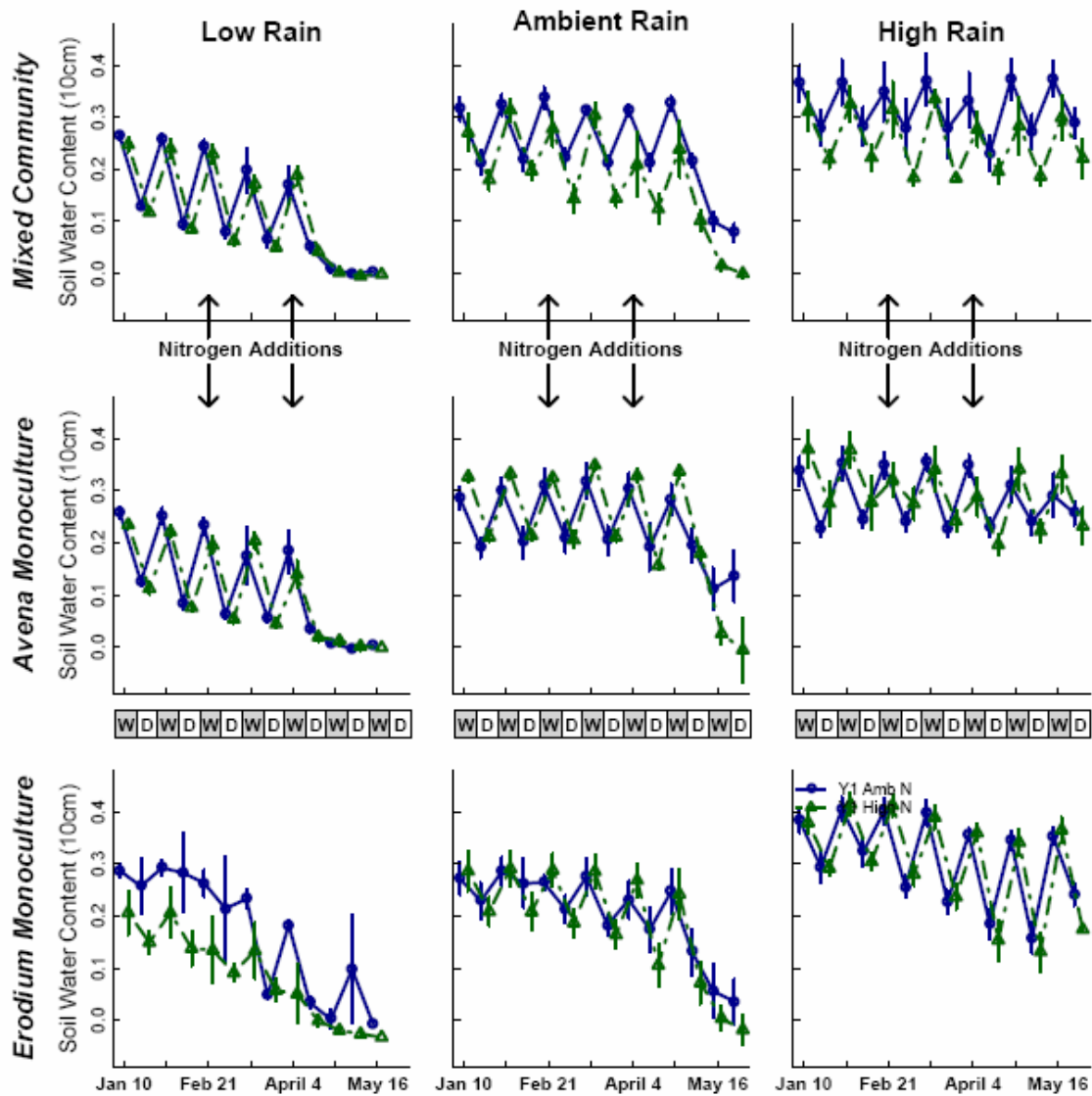


Figure 2. Soil moisture (10 cm depth) changes in response to treatment conditions during the 2006-2007 season. The “W” and “D” on the x-axis in the center of the plot indicate the wet and dry period of each cycle. Cycles 2-8 are shown in the figures. The blue line represents data from the ambient N plots and the green line indicates data points corresponding to N addition plots. Arrows indicate the two time points at which N additions were added. Symbols indicate mean values with the error bars representing SE.

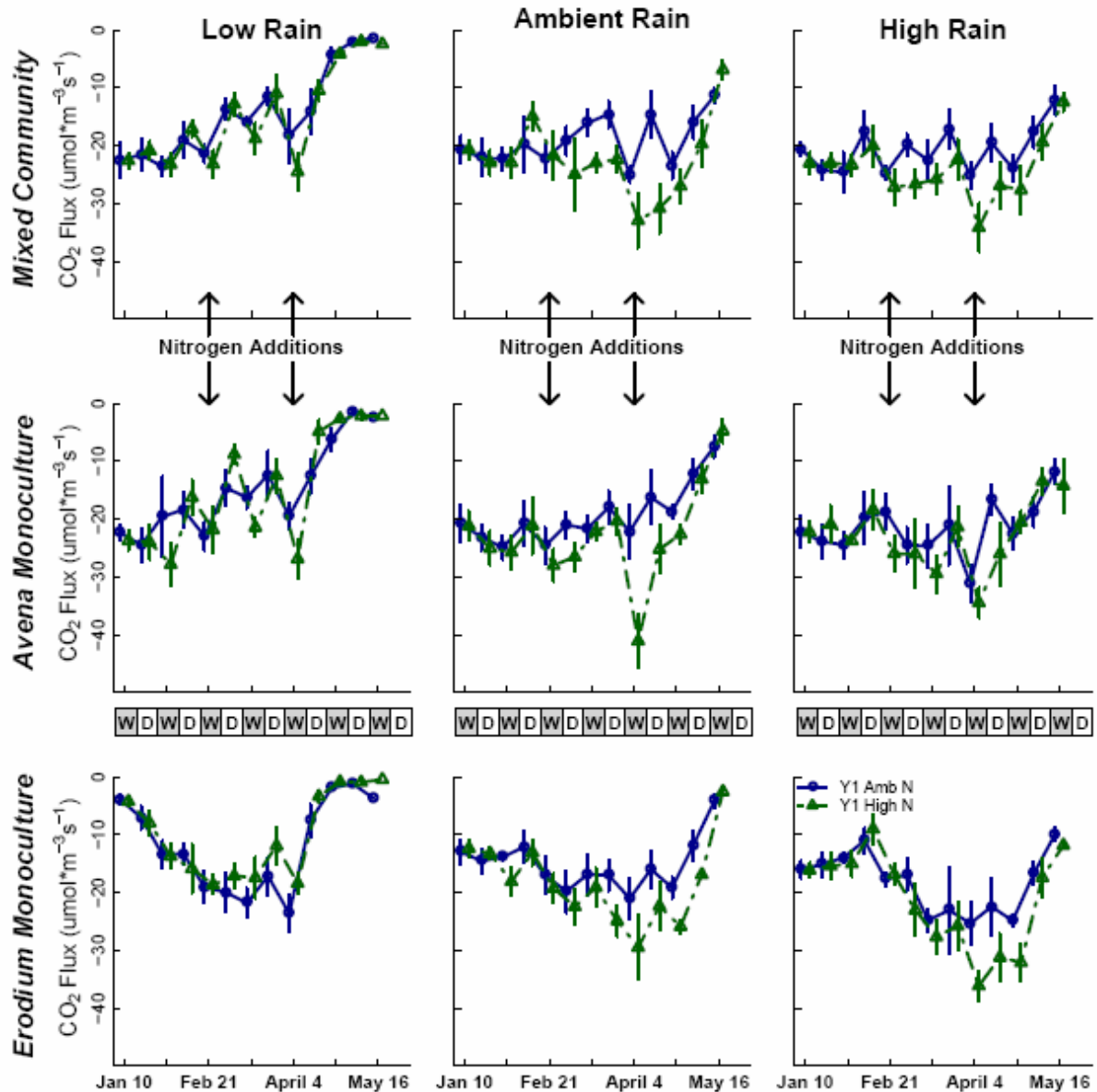


Figure 3. Net ecosystem CO₂ exchange in response to treatment conditions over the course of the 2006-2007 season. By standard convention more negative values represent greater ecosystem CO₂ fixation rates. Symbols indicate mean values with the error bars representing SE.

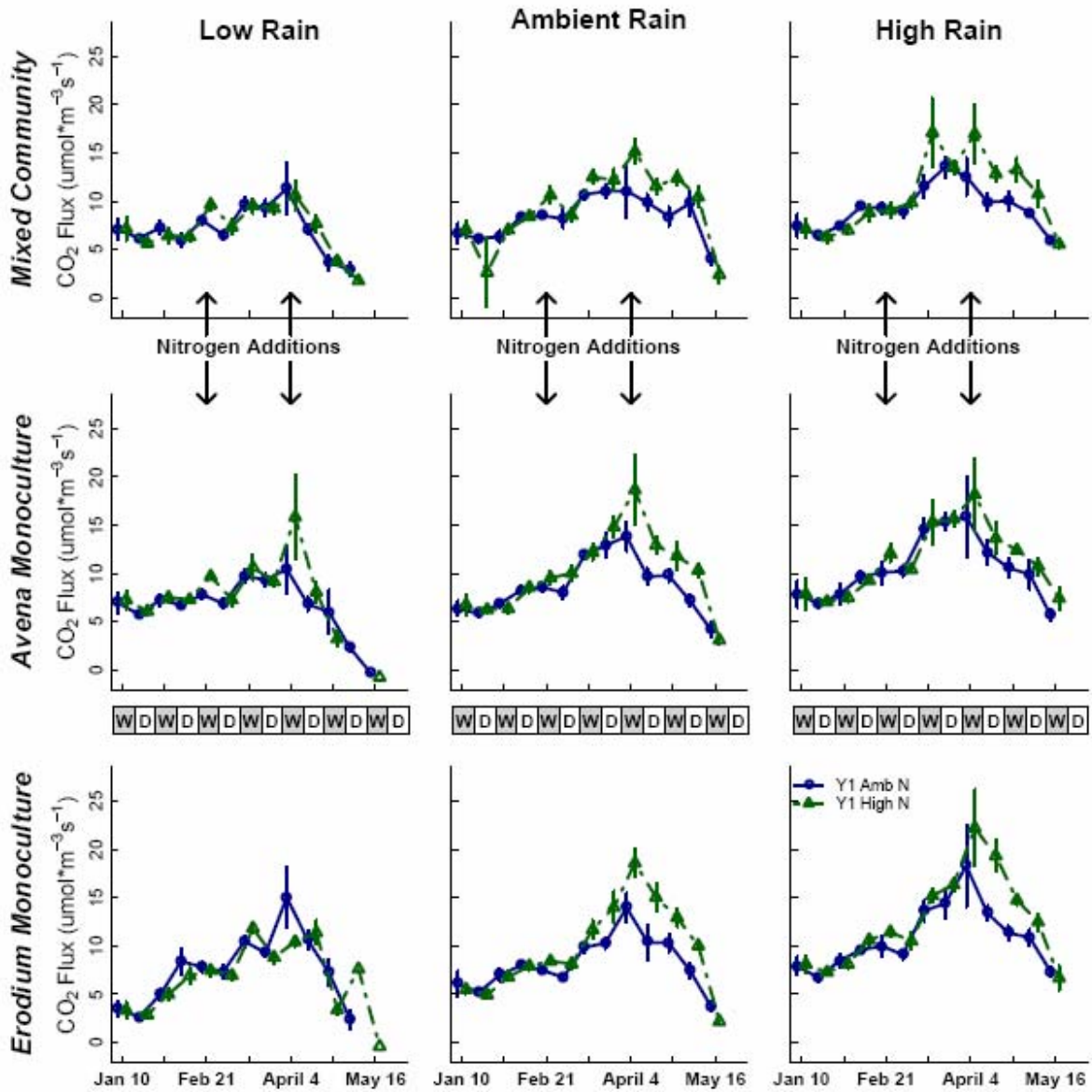


Figure 4. Ecosystem respiration in response to treatment conditions over the 2006-2007 season. Symbols indicate mean values with the error bars representing SE.

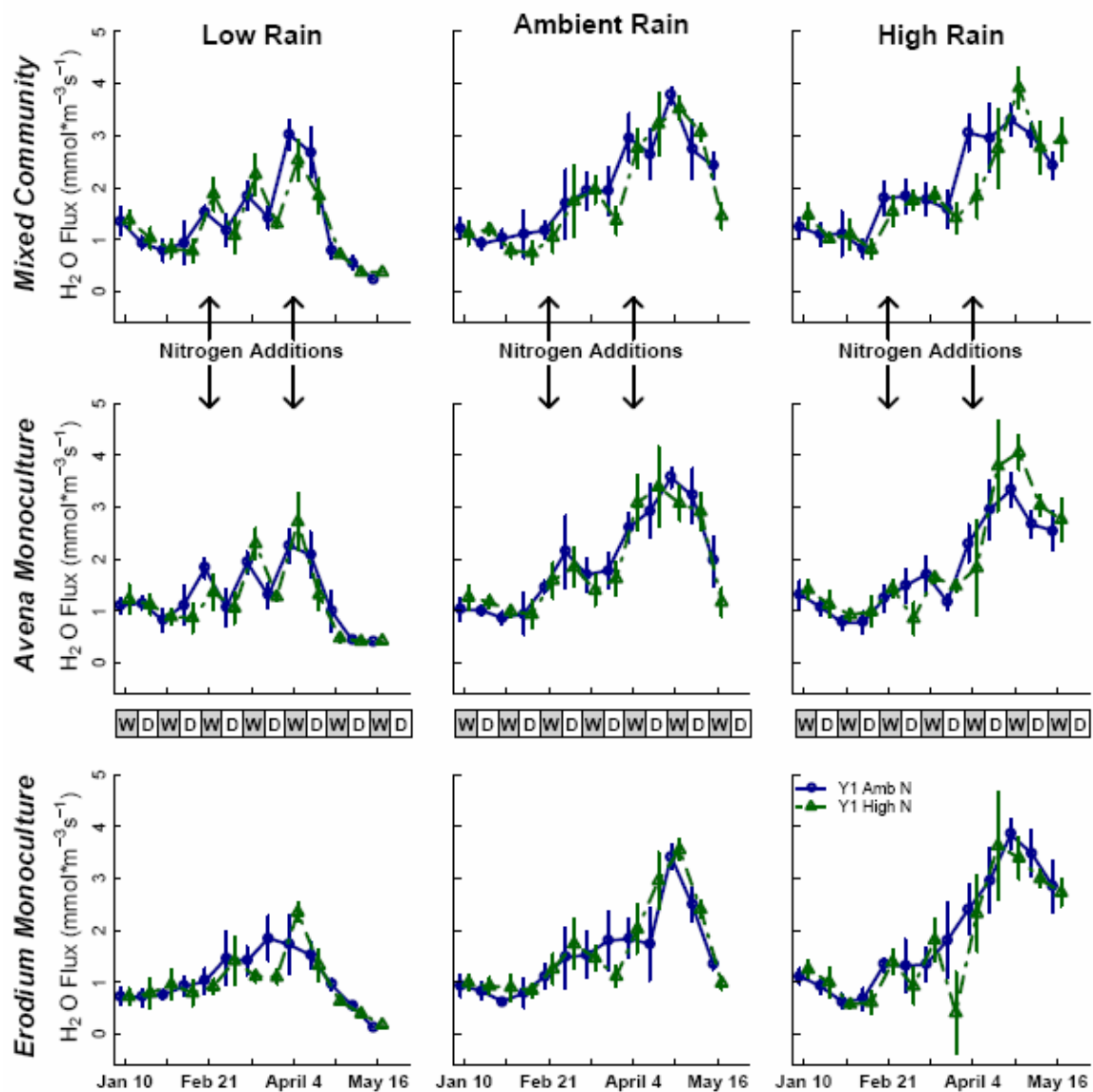


Figure 5. Ecosystem evapotranspiration rates in response to treatment conditions over the 2006-2007 season. Symbols indicate mean values with the error bars representing SE.

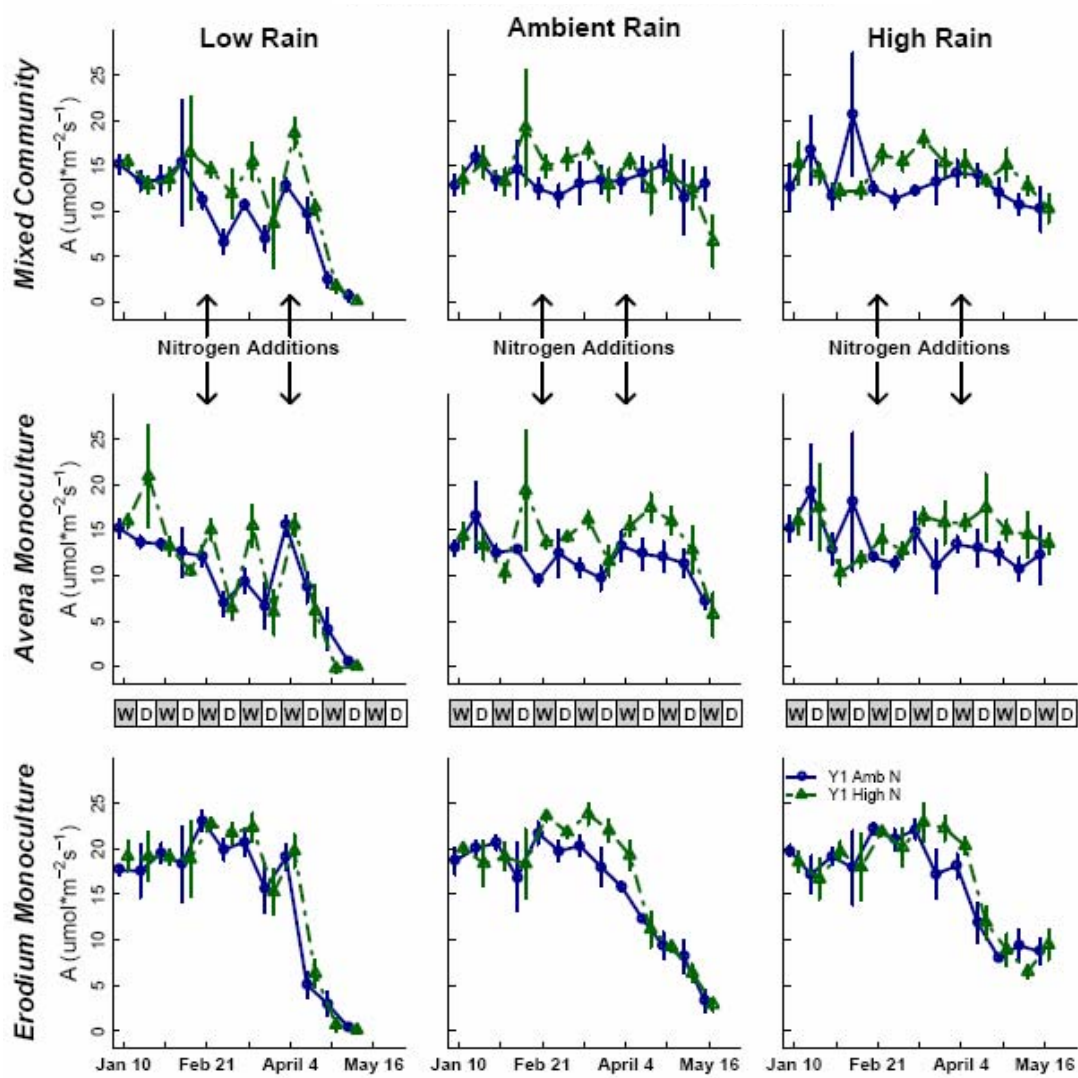


Figure 6. Leaf level photosynthesis responses to treatment conditions over the 2006-2007 season. Symbols indicate mean values with the error bars representing SE.