

# The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity

Kurt O. Reinhart · Sadikshya R. Dangi ·  
Lance T. Vermeire

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## Abstract

**Background and aims** Variation in fire intensity within an ecosystem is likely to moderate fire effects on plant and soil properties. We tested the effect of fire intensity on grassland biomass, soil microbial biomass, and soil nutrients. Additional tests determined plant-microbe, plant-nutrient, and microbe-nutrient associations.

**Methods** A replicated field experiment produced a fire intensity gradient. We measured plant and soil microbial biomasses at peak plant productivity the first growing season after fire. We concurrently measured flux in 11 soil nutrients and soil moisture.

**Results** Fire intensity positively affected soil nitrogen, phosphorus (P), and zinc but did not appreciably affect plant biomass, microbial biomass, and other soil nutrients. Plant biomass was seemingly (co-)limited by boron, manganese, and P. Microbial biomass was (co-)limited mainly by P and also iron.

**Conclusions** In the Northern Great Plains, plant and soil microbial biomasses were limited mainly by P and some

micronutrients. Fire intensity affected soil nutrients, however, pulsed P (due to fire) did not result in appreciable fire intensity effects on plant and microbial biomasses. Variable responses in plant productivity to fire are common and indicate the complexity of factors that regulate plant production after fire.

**Keywords** Co-limitation · Ecosystem management · Multiple regression · Rangeland · Semi-arid grassland

## Introduction

Fire is a common form of disturbance known to affect ecosystem functioning (Knapp et al. 1998; Neary et al. 1999; Wan et al. 2001). Fire intensity is likely to moderate the three main impacts of fire on grassland communities which include: 1) direct effect of heat on plants and soils, 2) removal of litter and standing biomass altering microclimates, and 3) effects on nutrients (Haile 2011; Mataix-Solera et al. 2009; Neary et al. 1999; Raison 1979). For example, intense fires are known to more fully combust aboveground biomass, decrease soil organic matter, and volatilize nutrients (Certini 2005; Hatten and Zabowski 2010). Fire intensity may vary by fire season, fuels, moisture, weather (Hamman et al. 2007; Neary et al. 1999; Wright and Bailey 1982), and is projected to increase with climate change (Pechony and Shindell 2010). While general fire effects per ecosystem are often well documented (Raison 1979; Wright and Bailey 1982), less is known about the role of fire intensity and whether variation in

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K. O. Reinhart (✉) · L. T. Vermeire  
United States Department of Agriculture- Agricultural Research Service, Fort Keogh Livestock & Range Research Laboratory, 243 Fort Keogh Road, Miles City, MT 59301-4016, USA  
e-mail: kurt.reinhart@ars.usda.gov

S. R. Dangi  
United States Department of Agriculture- Agricultural Research Service, Water Management Research Unit, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, CA 93648, USA

fire intensity within an ecosystem moderates fire effects on plant biomass, microbial biomass, and soil nutrients.

There is active interest in understanding the complex interactions between fire, nutrients, plants, and soil microbes (Reed et al. 2011). In a broad sense, soil microbes are beneficial to plants—microbial biomass and diversity are often positively correlated with plant productivity (Zak et al. 1994). Yet soil microbes may have negative (e.g., pathogenic) and complex interactions with plants and soil (review by Ehrenfeld et al. 2005). Soil microbes may affect nutrient cycling and nutrient mobility (review by Ehrenfeld et al. 2005). For example, the phosphorus (P) released from grassland fires may prime and increase nitrogen (N) fixation (Eisele et al. 1990; but see Hobbs and Schimel 1984). Soil microbes may also compete with plants for the same limiting nutrients (e.g., Bardgett et al. 2003; Dijkstra et al. 2012; Kuzyakov and Xu 2013). In grasslands, effects of fire on soil microbial biomass range from positive to negative (Dangi et al. 2010; Docherty et al. 2012; Garcia and Rice 1994; Harris et al. 2007; Picone et al. 2003). No study to date, however, has compared microbial properties across gradients of fire intensity in the same (grassland) ecosystem (Dooley and Treseder 2012).

In the expansive (>22 million ha) mixed-grass prairie of the Northern Great Plains, grassland dynamics are heavily influenced by precipitation, grazing, and fire (Anderson 2006; Oesterheld et al. 1999; Vermeire et al. 2009). Spring precipitation (April and May) is a main predictor of plant productivity in the region (Wiles et al. 2011). Here fire is often described as having negative effects on vegetation (Scheintaub et al. 2009; Wright and Bailey 1982 and citations therein). For example, total vegetation biomass and current year's biomass were reduced for several years following wildfires (Wright and Bailey 1982 and citations therein). But previous fire ecology research at the focal research station indicate that prescribed summer fires had modest effects on vegetation composition (Vermeire et al. 2011, 2014), plant density, and belowground axillary bud mortality (Russell et al. 2015). For example, fire increased  $C_3$  perennial grass biomass and reduced annual grass biomass (mainly exotic *Bromus* spp.), which had offsetting effects on total annual productivity. Summer fires reduced total biomass by removing past year's biomass, but did not affect annual biomass production (Vermeire et al. 2011). In this system, fire effects appear stochastic and can be delayed (i.e., appear second growing season post-fire). Science-based information on fire

effects for this system may inform land management policy, improve sustainability, and have direct economic and agricultural impacts on this important ecosystem. Efficacious fire management, however, requires understanding fire effects on total nutrient capital and nutrient dynamics.

We used a replicated field experiment to 1) test the effect of fire intensity on plant and soil properties and 2) used the variability in the dataset to uncover the nutrient(s) that limit biomass production by plants and soil microbes. We hypothesized that low to moderate intensity grassland fires, where fuel loads are typically  $< 3000 \text{ kg} \times \text{ha}^{-1}$  (8 years average =  $1607 \text{ kg} \times \text{ha}^{-1}$ ), would positively affect soil nutrients (e.g., N and P) and positively affect plant and microbial biomasses the first growing season after fire disturbance. We predicted P would increase along the fire disturbance gradient. We also predicted that plot-to-plot variation in available P would be positively associated with biomass production. This prediction was predicated by the knowledge that soils in many temperate grasslands, shrublands, and savannahs have B horizons that contain calcium carbonates (Palm et al. 2007) which may immobilize P (Ehrenfeld et al. 2005; Jones et al. 2013). Furthermore, a global nutrient addition experiment indicated that P often limited grassland productivity (Fay et al. 2015). To accomplish this, we sampled soil moisture, available soil nutrients, and biomass of 24 plots from an existing field experiment (eight burn treatments,  $n = 3$ ). We characterized available soil nutrients with ion exchange resins and total soil microbial biomass with phospholipid-derived fatty acids (PLFA, lipid biomarkers) (Buyer and Sasser 2012; Joergensen and Wichern 2008). Relatively few studies to date have used PLFA to quantify fire effects on soil microbial communities (Dooley and Treseder 2012 and citations therein), especially in grasslands (but see Antonsen and Olsson 2005; Dangi et al. 2010; Docherty et al. 2012). The full soil nutrient dataset was also used to determine the best predictors of variation in plant biomass, soil surface total microbial biomass (0–5 cm soil depth), and subsurface (5–15 cm) total microbial biomass at peak plant production. We interpreted that positive associations between an available nutrient and biomass seemingly indicate that the nutrient limited production. Our dataset enabled detecting fire intensity effects and biomass-nutrient associational patterns for 11 nutrients. Our fire intensity gradient includes a high level of realism (e.g., plots had head fires) because the experimental gradient is

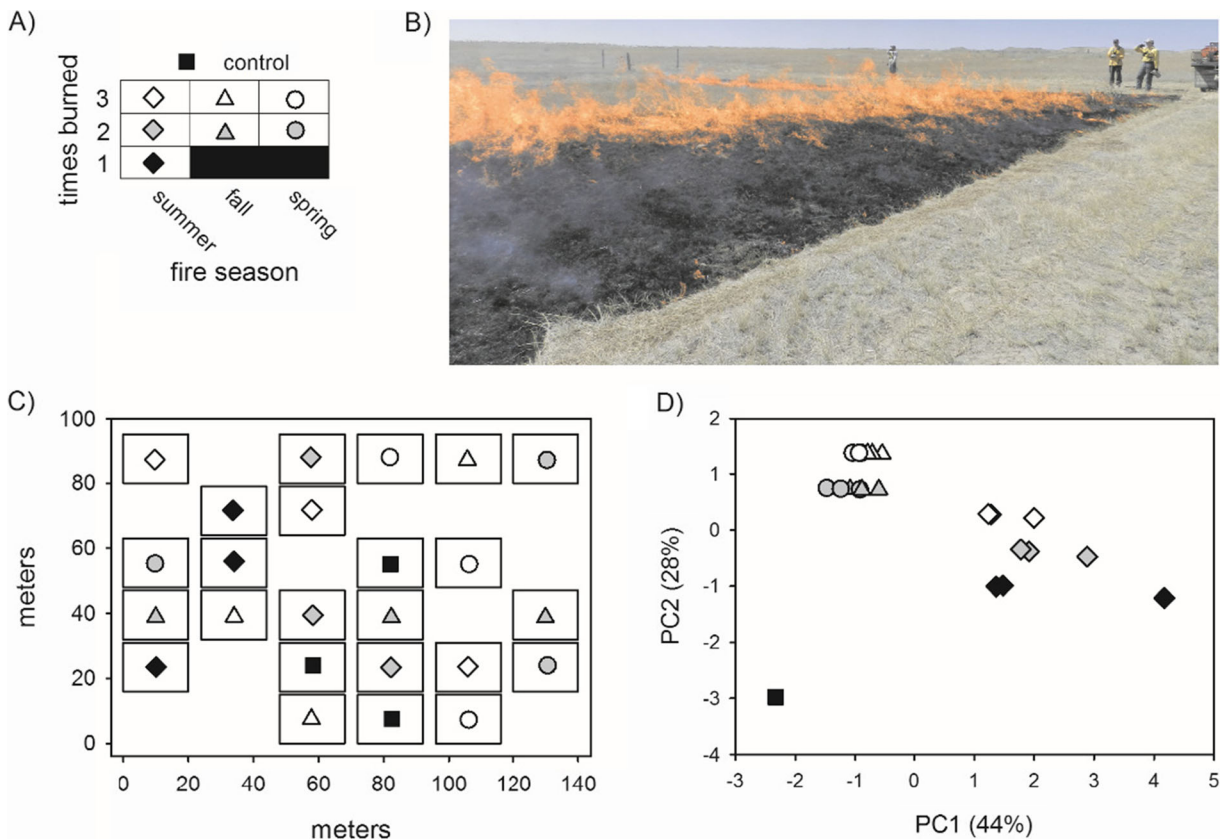
representative of variability due to rangeland management (e.g., fall or spring prescribed fire) and natural disturbances (i.e., summer wildfire) in the expansive Northern Great Plains.

**Methods**

**Study system and fire experiment**

Sampling was conducted on a multiyear (2006 to present) fire ecology field experiment (Fig. 1) established on a loamy ecological site at the Fort Keogh Livestock and Range Research Laboratory in eastern Montana, USA. The study site (46°23'59.90"N, 105°57'1.23"W) was at an elevation of 794 m, has a gentle slope, and has loamy soil (Pinehill loam [81 %] and Pinehill-Absher complex [19 %], frigid Leptic Torrertic Natrustalfs and frigid Aridic Haplustalfs). The grassland is a “calcareous grassland” since its B horizon contains calcium

carbonates. The site is centrally located in the Northern Great Plains Steppe ecoregion (unglaciated portion) of North America where grasslands persist on more than 22 million ha and are dominated by mixed-grass prairie vegetation (Martin et al. 1998). The dominant grasses are *Pascopyrum smithii*, *Hesperostipa comata*, and *Bouteloua gracilis*. Peak annual productivity (Table 1) for this system typically occurs between June and July (Vermeire et al. 2009) and is limited mainly by April and May precipitation (Wiles et al. 2011). April and May precipitation was above-average (243 % of 73-yr average in 2013 [202 vs. 83 mm]) in the sampled year but was below-average the year before (35 % of average in 2012 [29 vs. 83 mm]). Furthermore, soil moisture in 2013 was either not correlated ( $r = 0.08$ ,  $P = 0.69$ ) or negatively correlated ( $r = -0.42$ ,  $P = 0.043$ ) with plant production which indicates that plant production was likely not limited by moisture in 2013. (One interpretation is that plots with greater peak biomass had greater levels of transpiration and actually reduced levels of



**Fig. 1** Fire treatments and symbol key (a), photograph showing a prescribed fire at the field site (b), map of experimental plots (c), and principal component analysis (PCA) of fire properties (d). The

field experiment included plots with varying fire histories (burned once, twice, or thrice in a 7 years period) and seasons (n = 3). (photo credit: Morgan Russell)

**Table 1** Average (minimum, maximum) biomasses, fire intensity, soil nutrients, soil pH, and soil moisture for the studied grassland

Site properties	Average (min., max.)
<b>Biomasses</b>	
plant	1333 (877, 1966)
microbial (0–5 cm)	5.7 (3.4, 8.8)
microbial (5–15 cm)	3.4 (2.0, 5.1)
<b>Fire intensity</b>	
heat dosage	8589 (0, 25880)
heat duration	174 (0, 877)
<b>Soil nutrients</b>	
B	1.3 (0.1, 2.9)
Ca	1957 (1541, 2498)
Cu	0.4 (0.2, 0.7)
Fe	7.7 (4.2, 12.9)
K	96 (53, 166)
Mg	485 (412, 606)
Mn	3.6 (1.3, 9.8)
N	45 (8, 130)
P	19 (7, 29)
S	55 (26, 102)
Zn	0.9 (0.5, 1.5)
<b>Soil pH and moisture</b>	
pH	7.0 (6.3, 7.7)
Sm1	0.18 (0.15, 0.21)
Sm2	0.33 (0.25, 0.40)

Units: peak annual plant biomass ( $\text{kg} \times \text{ha}^{-1}$ ), microbial biomass ( $\mu\text{g} \times \text{g soil}^{-1}$ ), heat dosage ( $^{\circ}\text{C} \times \text{second}^{-1}$ ), heat duration (seconds  $>60^{\circ}\text{C}$ ), and soil nutrients ( $\mu\text{g}$  of nutrient  $\times 10 \text{ cm}^{-2} \times \text{burial time}^{-1}$ ). Soil nutrients include: B = boron, Ca = calcium, Cu = copper, Fe = iron, K = potassium, Mg = magnesium, Mn = manganese, N = nitrogen (nitrate and ammonium), P = phosphorus, S = sulfur, and Zn = zinc. Volumetric soil moisture (%) includes: Sm1 = surface soil moisture (7.6 cm) and Sm2 = subsurface soil moisture (20 cm)

subsurface soil moisture relative to plots with less production.)

In 2013, 24, 300-m<sup>2</sup> plots treated with eight different fire season and history treatments ( $n = 3$ ) were sampled (Fig. 1). Summer fire treatments included: non-burned controls and plots burned once, twice, and thrice in a 7 year period (2006–2013). Prior to the sampling in 2013, all summer fire treatments were last burned 16 August 2012 (while the research station and region experienced a drought and wildfires). Non-burned control plots were assumed to have not experienced a wildfire in  $> 20$  yrs. The remaining plots included fall (after

first  $-2^{\circ}\text{C}$  frost) and spring (after the *C*<sub>4</sub> grass *Bouteloua gracilis* initiates growth) fire treatment plots burned twice and thrice in a seven yr period (2006–2013) and prior to sampling were last burned 19 October 2012 and 6 May 2013, respectively.

#### Fire properties

To account for varying fire conditions and behavior per plot, time-temperature profiles were created for each plot with HOBO™ Thermocouple Data Loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) with K-type Thermocouples (Omega Engineering, Inc., Stamford, Connecticut, USA). Thermocouples were placed in each plot within the crown of a target plant (10 thermocouples  $\times$  plot<sup>-1</sup>) and data loggers were programmed to record temperatures at one-second intervals. These data were used to quantify heat duration (seconds  $>60^{\circ}\text{C}$ ) and heat dosage which is the sum of the products of time and degrees  $>60^{\circ}\text{C} \times \text{s}$  (degree-seconds). Under highly controlled conditions, fuel load (i.e.,  $\text{kg}$  of plant biomass  $\times \text{ha}^{-1}$ ) is positively correlated with heat dosage ( $R^2 = 0.58$ ) and heat duration ( $R^2 = 0.36$ ) (Haile 2011).

Final fire properties per plot included: fire season (converted into dummy variables), times burned in 7 years, heat dosage, and heat duration. Dimensionality of these fire properties (e.g., Delgado-Baquerizo et al. 2013) was reduced with a principal component analysis using the vegan package (Oksanen et al. 2015) in R (R Development Core Team 2011). This reduced the fire properties to principal components. The sign of the first principal component (PC1) was then changed so that it was positively associated with two measures of fire intensity (Fig. 1d)—heat dosage ( $R^2 = 0.94$ ) and heat duration ( $R^2 = 0.84$ ). The first component from the PCA was kept for further analyses, which had an eigenvalue of 3.1 and explained 44 % of the variance from the PCA (Fig. 1d). Here the gradient includes plots with varying fire histories and seasons burned and variability due to edaphic factors.

#### Plant and soil microbial biomasses

In four 0.25 m<sup>2</sup> quadrats per treatment plot, above-ground biomass was clipped at ground level in early July (2013) when regional grasslands were at peak productivity. Plant material was dried to constant weight, separated into current-year and older material,

and weighed. Current year biomass was used to measure peak annual plant productivity.

At peak plant biomass, living soil microbial biomass was determined by PLFA biomarkers. Soil samples for PLFA were collected on 11 July 2013, the growing season immediately after fire. Soil was sampled near the surface (0–5 cm) and subsurface (5–15 cm). Soil was cored from four random positions along each of three transects per plot, and samples were aggregated per depth per transect. Aggregated samples were homogenized to yield three soil subsamples per depth per plot (3 transects  $\times$  2 depths  $\times$  24 plots = 144 samples). The soil probe was flame sterilized between plots. Subsamples per plot were sealed in a plastic bag; stored on dry ice immediately after collection, transported to the laboratory, and stored at -20 °C; then shipped on dry ice to USDA-ARS-SJVASC, Parlier, CA; and stored in a -20 °C freezer until analyzed (e.g., Dangi et al. 2010, 2012).

Phospholipid-derived fatty acids were extracted from 5 g soil samples using a modified Bligh-Dyer method (Buyer et al. 2010). Fatty acids were directly extracted from soil samples using a mixture of chloroform: methanol: phosphate buffer (1:2:0.8). Phospholipid-derived fatty acids were separated from neutral and glycolipid fatty acids in a solid phase extraction column. After mild alkaline methanolysis, PLFA samples were qualitatively and quantitatively analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA). Fatty acids were identified using the MIDI PLFAD1 calibration mix and naming table (Buyer and Sasser 2012; MIDI Inc., Newark, NJ).

Individual PLFA signatures were used to quantify abundance of specific microbial groups in soil samples (see Buyer and Sasser 2012). Eukaryotes were identified and quantified by the presence of polyunsaturated fatty acids, Gram positive bacteria were identified and quantified by presence of iso- and anteiso-branched fatty acids, Gram negative bacteria with monounsaturated fatty acids and cyclopropyl 17:0 and 19:0, and eubacteria with 15:0, 17:0 cyclo, 19:0 cyclo, 15:1 iso, 17:1 iso and 17:1 anteiso. Fungi were identified and quantified with 18:2  $\omega$ 6c, anaerobes with Dimethyl acetal (DMA), and actinomycetes with 10-methyl fatty acids (Blackwood and Buyer 2004; Cavigelli et al. 1995; Frostegård et al. 1993; Zelles et al. 1994, 1995). Total PLFA (i.e., sum of specified PLFA lipid biomarkers,  $\mu\text{g} \times \text{g soil}^{-1}$ ) was used as a measure of microbial biomass (Buyer et al. 2010). Variation in microbial

communities was also tested; however, after accounting for spatial variability, microbial community structure was not appreciably related to plant, fire, and soil nutrients (Kurt O. Reinhart, *unpublished results*).

#### Soil moisture and nutrient concentrations

Since precipitation is a main determinant of annual net primary productivity (Wiles et al. 2011), volumetric soil moisture was measured with a Field Scout TDR-100 (Spectrum Technologies, Inc., Aurora, IL) bi-weekly 1 April–1 November. In each plot, soil moisture was measured in quadruplicate from random points at two depths (7.6 and 20 cm). Data were averaged for the time period (see below) ion exchange resins were deployed in the field.

Soil nutrient concentrations of boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), N (nitrate and ammonium), P, potassium (K), sulfur (S), and zinc (Zn) were estimated using pairs of cation and anion ion exchange probes (Plant Root Simulator™-probes [PRST™ probes], Western Ag Innovations, Saskatoon, Saskatchewan, Canada). PRST™ probes provide an integrative measure of soil nutrient pools during the period for which the probes are in the soil (e.g., Hanks et al. 2004). Ion exchange resins are one useful approach to uncover nutrient limitation (Chapin et al. 1986). Each probe contained a single 17.5-cm<sup>2</sup> resin membrane, which was placed vertically, between 2 and 7.6 cm below the soil surface on 10 May 2013 and extracted 11 July 2013. Others have used similar approaches to quantify available nutrients *in situ* (e.g., Augustine et al. 2014; Johnson et al. 2014; Jones et al. 2013). In such studies, the probes were not isolated from plant roots—plant roots may compete with the ion resins for nutrients. An alternative approach is to eliminate roots by establishing root exclusion chambers and then insert PRST™ probes directly into the chambers (Hanks et al. 2004). [However, such chambers may have unintended effects (e.g., have gaps between the chamber wall and soil that enable ash, where present, to rapidly migrate to the soil subsurface and/or plastic walls may melt in response to various fire treatments).] Since roots were not excluded from around PRST™ probes, estimates of total nutrient levels are likely conservative. The probe insertion period was intended to capture the nutrient fluxes for the 2 months preceding our measurements of (peak) annual plant productivity and concurrent microbial productivity (see *Discussion* section for

caveats). To minimize effects of small-scale spatial variation within a plot in detectable nutrients, three sets of four pairs of probes, each comprised of one cation and one anion probe, were inserted randomly within each plot (24 plots  $\times$  3 probe sets  $\times$  4 probe pairs per set  $\times$  2 probes per pair = 579 total probes). Probes were cleaned with deionized water immediately after removal from the soil, and shipped to Western Ag Innovations. Probes were then extracted with 0.5 M HCl and analyzed colorimetrically with an autoanalyzer to determine nutrient concentrations.

## Analyses

Data summaries are provided (Table 1). To visualize the (dis)similarities in fire properties among the 24 plots, a PCA analysis was performed (see *Fire properties* above). Appropriate regression models (linear or non-linear) were then identified and tested to determine whether fire intensity (PC1) affected plant biomass, microbial biomass, and soil nutrients. After checking for normality and homogeneity of residual variances, some data were natural log transformed.

Multiple linear regression (MLR) was also used to elucidate the soil nutrient variables which explained the greatest variation in plant and microbial biomasses. MLRs were performed separately for surface (0–5 cm) and subsurface (5–15 cm) microbial biomasses. The `regsubsets` function in the `leaps` package (Lumley and Miller 2009) in R was used with the exhaustive search method to determine the three best models per level of parameters. `Leaps` uses an efficient branch-and-bound algorithm to rapidly determine the best models. The best model per MLR was identified using Schwarz's Bayesian information criterion. Reported are traditional parametric statistics for models and the relative importance (RI) of each parameter based on the `lmg` function in the `relaimpo` package (Grömping 2007) which averages sequential sums of squares over all orderings of regressors. RI values sum to the total  $R^2$ . Residual analyses included visual confirmation that the assumptions of normality and homoscedasticity of residuals were not violated. Many soil nutrients were linearly correlated (Table 2), which raised concerns of multicollinearity among variables. Multicollinearity among parameters was assessed in selected models with condition index scores. To help visualize the relative importance of variables, partial regressions were plotted.

## Results

In the studied grassland, fire intensity had no appreciable effect on plant biomass ( $P = 0.44$ ) and soil microbial biomasses ( $P \geq 0.12$ , Table 2). Yet increasing fire intensity increased available soil N, P, and Zn (Fig. 2). As fire intensity increased, available N increased but then plateaued (Fig. 2). Phosphorus and Zn increased linearly across the range of fire intensities. Across the experimental site, we determined that a single axis of spatial distance (Table 2) explained significant variation in plant biomass, subsurface microbial biomass (5–15 cm), B, K, P, and subsurface soil moisture (20 cm) (see caveat section in Discussion). We also determined that the spatial axis-plant productivity relationship occurred in several other years including 2007 ( $R^2 = 0.18$ ,  $n = 36$ ,  $P = 0.01$ ), 2009 ( $R^2 = 0.23$ ,  $n = 36$ ,  $P = 0.01$ ), and 2013 ( $R^2 = 0.29$ ,  $n = 36$ ,  $P < 0.001$ ) and a marginally significant ( $\alpha \leq 0.10$ ) association was detected for 2011.

Peak annual plant biomass was positively correlated with subsurface microbial biomass and three soil nutrients (B, Mn, and P; Table 2). Of these three nutrients, two (B and P) varied by spatial distance, and one (P) varied by fire intensity (Table 2). Interestingly, peak plant biomass was negatively correlated with subsurface soil moisture (Table 2) indicating that areas with greater biomass had lower levels of subsurface soil moisture (20 cm) than expected. It is worth reminding the reader that in the sampling year, precipitation was above-average (243 % of the historic average). Subsurface soil moisture was also negatively correlated with several soil nutrients (K, Mg, and P), especially P (Table 2).

Variation in surface and subsurface microbial biomasses was largely unexplained and was not individually correlated with any of the 11 soil nutrients nor soil moisture (Table 2). We did detect marginally significant ( $\alpha \leq 0.10$ ) positive associations between surface microbial biomass and Ca and subsurface microbial biomass and P (Table 2). Subsurface microbial biomass was associated ( $\alpha \leq 0.05$ ) with plant biomass and spatial distance (Table 2). Several soil nutrients were correlated indicating the potential for multicollinearity among subsets of soil nutrients (e.g., Zn was positively correlated with B, Cu, Fe, Mn, and S) (Table 2).

Multiple linear regressions (MLR) were used to determine plant-nutrient associations and microbe nutrient-associations and determined that moderate amounts of variation in plant and microbial biomasses were explained by variation in subsets of soil nutrients.

**Table 2** Pearson product–moment correlations (r) and p-values (in parentheses) for plant biomass (ANPP), microbial biomass (MB1, MB2), fire intensity (fire), spatial distance (dist), and soil

properties (nutrients and moisture) in a northern mixed-grass prairie. In bold are correlation coefficients with significant ( $\alpha \leq 0.05$ ) p-values

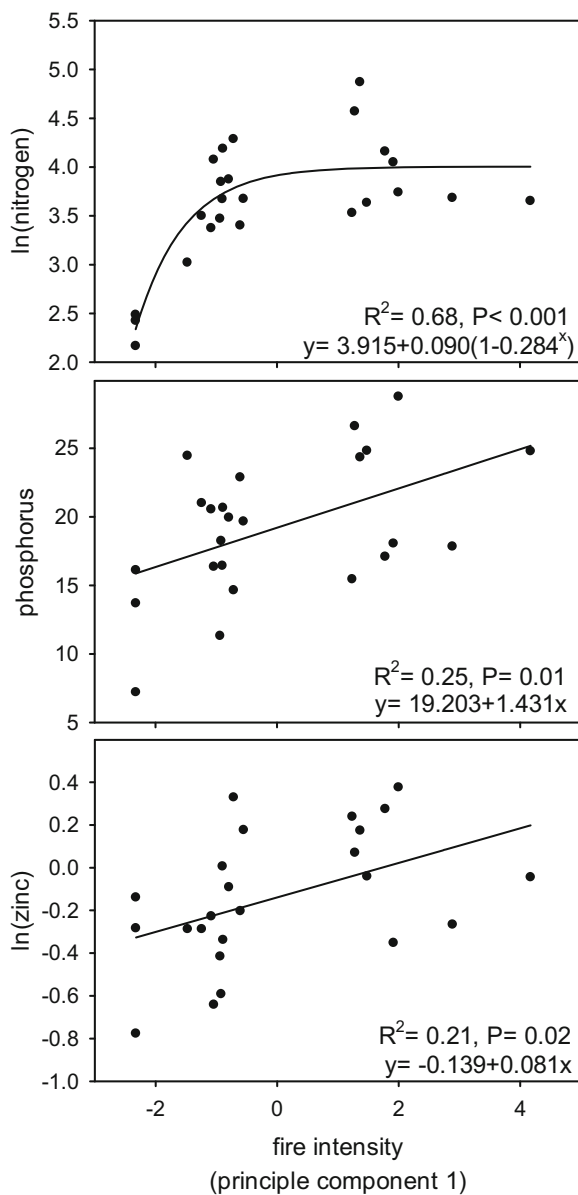
	ANPP	MB1	MB2	Fire	Dist	B	Ca	Cu	Fe
MB1	0.38 (0.070)	–	–	–	–	–	–	–	–
MB2	<b>0.43 (0.036)</b>	<b>0.74 (&lt;0.001)</b>	–	–	–	–	–	–	–
Fire	0.16 (0.44)	0.16 (0.46)	0.32 (0.12)	–	–	–	–	–	–
Dist	<b>–0.72 (&lt;0.001)</b>	–0.34 (0.099)	<b>–0.63 (&lt;0.001)</b>	–0.37 (0.071)	–	–	–	–	–
B	<b>0.44 (0.031)</b>	0.25 (0.23)	0.33 (0.11)	0.35 (0.097)	<b>–0.47 (0.020)</b>	–	–	–	–
Ca	–0.03 (0.88)	0.36 (0.089)	0.15 (0.49)	0.009 (0.97)	0.19 (0.38)	0.36 (0.082)	–	–	–
Cu	0.17 (0.42)	–0.08 (0.69)	–0.07 (0.74)	0.23 (0.28)	–0.02 (0.96)	<b>0.44 (0.033)</b>	0.16 (0.45)	–	–
Fe	0.30 (0.15)	–0.005 (0.98)	–0.06 (0.79)	0.32 (0.13)	–0.15 (0.50)	<b>0.45 (0.028)</b>	0.10 (0.64)	<b>0.80 (&lt;0.001)</b>	–
K	0.21 (0.33)	0.02 (0.93)	0.26 (0.22)	0.31 (0.14)	<b>–0.43 (0.035)</b>	0.14 (0.51)	–0.37 (0.072)	–0.35 (0.096)	–0.01 (0.95)
Mg	0.02 (0.94)	–0.37 (0.072)	–0.38 (0.065)	0.17 (0.42)	–0.03 (0.87)	–0.09 (0.68)	–0.34 (0.110)	0.37 (0.072)	<b>0.47 (0.021)</b>
Mn	<b>0.41 (0.047)</b>	–0.16 (0.46)	–0.20 (0.35)	0.30 (0.16)	–0.17 (0.42)	0.35 (0.096)	–0.003 (0.99)	<b>0.66 (&lt;0.001)</b>	<b>0.86 (&lt;0.001)</b>
N	0.19 (0.37)	–0.14 (0.53)	–0.01 (0.95)	<b>0.41 (0.049)</b>	–0.03 (0.89)	0.26 (0.21)	0.13 (0.55)	<b>0.41 (0.046)</b>	<b>0.62 (0.001)</b>
P	<b>0.43 (0.038)</b>	0.21 (0.32)	0.37 (0.079)	<b>0.49 (0.013)</b>	<b>–0.52 (0.010)</b>	0.19 (0.37)	–0.07 (0.74)	–0.15 (0.49)	0.25 (0.24)
S	–0.16 (0.45)	–0.02 (0.91)	–0.13 (0.55)	0.34 (0.10)	0.31 (0.14)	0.11 (0.62)	0.37 (0.079)	<b>0.47 (0.021)</b>	<b>0.56 (0.005)</b>
Zn	0.34 (0.10)	0.20 (0.35)	0.31 (0.14)	<b>0.44 (0.030)</b>	–0.40 (0.052)	<b>0.65 (&lt;0.001)</b>	0.23 (0.27)	<b>0.65 (&lt;0.001)</b>	<b>0.77 (&lt;0.001)</b>
Sm1	0.08 (0.69)	0.03 (0.90)	–0.008 (0.97)	–0.35 (0.095)	–0.10 (0.64)	0.25 (0.23)	0.39 (0.057)	–0.05 (0.82)	–0.21 (0.33)
Sm2	<b>–0.42 (0.043)</b>	–0.14 (0.52)	–0.34 (0.11)	–0.31 (0.14)	<b>0.48 (0.017)</b>	–0.05 (0.83)	0.35 (0.091)	0.11 (0.61)	–0.18 (0.40)
	K	Mg	Mn	N	P	S	Zn	Sm1	
Mg	0.13 (0.54)	–	–	–	–	–	–	–	–
Mn	0.06 (0.76)	<b>0.51 (0.011)</b>	–	–	–	–	–	–	–
N	0.01 (0.99)	0.09 (0.67)	<b>0.66 (&lt;0.001)</b>	–	–	–	–	–	–
P	<b>0.61 (0.002)</b>	0.31 (0.14)	0.32 (0.13)	0.37 (0.072)	–	–	–	–	–
S	–0.19 (0.37)	0.19 (0.36)	<b>0.42 (0.043)</b>	<b>0.55 (0.006)</b>	0.09 (0.67)	–	–	–	–
Zn	0.17 (0.43)	0.29 (0.17)	<b>0.61 (0.002)</b>	0.39 (0.058)	0.37 (0.075)	<b>0.45 (0.028)</b>	–	–	–
Sm1	–0.35 (0.09)	<b>–0.52 (0.009)</b>	–0.15 (0.47)	–0.15 (0.48)	<b>–0.44 (0.033)</b>	–0.33 (0.12)	–0.13 (0.56)	–	–
Sm2	<b>–0.60 (0.002)</b>	<b>–0.46 (0.023)</b>	–0.22 (0.31)	–0.04 (0.87)	<b>–0.80 (&lt;0.001)</b>	–0.03 (0.88)	–0.36 (0.08)	<b>0.62 (0.001)</b>	–

ANPP = peak annual plant biomass in 2013, MB1 = surface (0–5 cm) microbial biomass 2013, MB2 = subsurface (5–15 cm) microbial biomass 2013. Fire = principal component 1 for fire intensity gradient of prescribed fire treatments in 2012 and 2013 (see Fig. 1), and Dist = plot position (m) along the longest length of the rectangular field experiment (Fig. 1c). Soil nutrient and soil moisture (Sm) abbreviations were defined in Table 1

Specifically, variation in plant biomass was best explained by a MLR ( $F_{2,21} = 4.86, P = 0.019, R^2 = 0.32$ ) (Table 3) that included the parameters B and P. Both soil nutrients were positively associated with plant biomass. A comparison of their relative importance scores (Table 3) indicate that they explained nearly equal amounts of variation. We plotted partial regression plots (Fig. 3) to help visualize the (positive) associations and the residual variance of each parameter after controlling for the influence of all other model variables.

At the soil surface (0–5 cm), a moderate amount of variation in total microbial biomass was best explained by a MLR ( $F_{4,19} = 4.54, P = 0.010, R^2 = 0.49$ ) (Table 4) that included the parameters: Fe, Mg, N, and P. The

MLR model indicated that soil surface microbial biomass was positively associated with Fe and P (negatively associated with Mg and N). The relative importance values indicate that variation in surface soil microbial biomass was mainly due to Mg and then P. At the soil subsurface (5–15 cm), a moderate amount of variation in total microbial biomass was best explained by a MLR ( $F_{4,19} = 4.79, P < 0.008, R^2 = 0.50$ ) (Table 4, MB<sub>5-15</sub>) that also included the parameters: Fe, Mg, N, and P. The relative importance values indicate that variation in subsurface soil microbial biomass was mainly due to Mg and then P. Partial regression plots further indicate the positive association between subsurface microbial biomass and Fe and P and the residual variance of each parameter (Fig. 4).



**Fig. 2** Positive effects of fire intensity (principle component 1) on soil nitrogen, phosphorus, and zinc ( $\mu\text{g}$  of nutrient  $\times 10 \text{ cm}^{-2} \times$  burial time $^{-1}$ ). Equations, best-fit lines, goodness of fit ( $R^2$ ), and p-values are provided

## Discussion

### i) Fire intensity effects

Fire intensity may moderate the three main impacts of fire on grassland communities including: 1) direct effect of heat on plants and soils, 2) removal of litter and standing biomass altering microclimates, and 3) effects on nutrients (Haile 2011; Mataix-Solera et al. 2009;

Neary et al. 1999; Raison 1979). In our grassland experiment, we observed no appreciable effect of fire intensity on peak annual plant biomass. The vegetation consists predominately of perennial grasses which are highly resistant to fire (Haile 2011). A study by Haile (2011) determined the fuel loads and heat dosages necessary to kill 50 % of two regionally dominant grasses (*Bouteloua gracilis* and *Hesperostipa comata*). From Haile's (2011) work, we can interpret that our maximum fuel loads ( $\leq 1,966 \text{ kg} \times \text{ha}^{-1}$ , Table 1) and heat dosages ( $\leq 25,880 \text{ }^\circ\text{C} \times \text{second}^{-1}$ ) were too low to directly cause appreciable plant mortality—50 % mortality predicted with fuel loads  $\geq 8,000 \text{ kg} \times \text{ha}^{-1}$  or heat dosage  $\geq 44,230 \text{ }^\circ\text{C} \times \text{second}^{-1}$ . Though peak living biomass was unaffected by fire intensity, previous work has shown that some functional groups, such as annual grasses, shrubs, and cacti, are susceptible to fire (e.g., Vermeire et al. 2011, 2014). Though burned areas may have higher levels of limiting nutrients, variable responses in plant productivity are common and indicate the complexity of factors that regulate plant production after fire (Raison 1979; Wright and Bailey 1982). This variability is partly due to among study variability in treatments and contexts. For example, a study may quantify different results when measuring plant recovery from fires applied in wet versus dry years.

From a global meta-analysis, fire reduced microbial biomass by 33 % across all biomes but effects were inconsistent among biomes (i.e., forests vs. grasslands) and fire types (e.g., wildfire vs. prescribed fires) (Dooley and Treseder 2012). Wildfires reduced microbial biomass but prescribed fires did not (Dooley and Treseder 2012). In systems with greater fire intensity (e.g., forests and shrublands), microbial biomass was reduced by fire (Dooley and Treseder 2012; Holden and Treseder 2013). We found that fire intensity had no appreciable impact on surface (0–5 cm) and subsurface (5–15 cm) soil microbial biomasses the first growing season after fire treatments. The maximum fire intensity observed in our experiment was too low to directly impact soil microbial biomass in the soil subsurface (below 2 cm depth). Specifically, simulated fuel loads ( $4,100 \text{ kg} \times \text{ha}^{-1}$ ), nearly twice that observed in our study, had no appreciable effect on soil temperature 2 cm below the surface (Branson and Vermeire 2007). In grasslands with greater fuel loads, fires are more likely to cause more substantive soil heating. Here the most intense fires occurred in the summer (Fig. 1) and during drought. Wildfire risk is positively



**Table 3** Best multiple regression model, based on Schwarz Bayesian Criterion scores, to explain variation in peak annual plant biomass (plant)

Dependent variables	Independent variables	Beta coefficients	t-value	$F_{2,21}$	P	$R^2$	RI
plant	B	0.37	2.03	–	0.055	–	0.52
	P	0.36	1.93	–	0.067	–	0.48
	Total	–	–	4.86	0.019	0.32	–

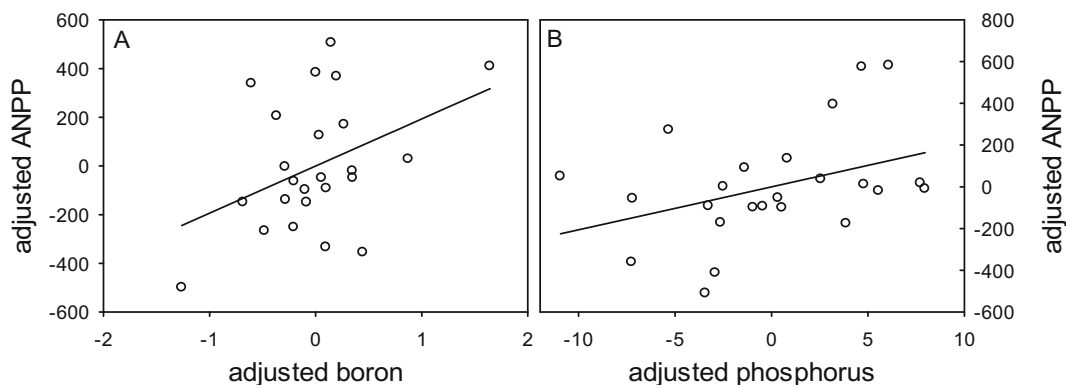
plant = peak annual biomass in 2013 ( $\text{kg} \times \text{ha}^{-1}$ ) and RI = relative importance (%). Variables included the following soil nutrients: B = boron and P = phosphorus ( $\mu\text{g}$  of nutrient  $\times 10 \text{ cm}^{-2} \times \text{burial time}^{-1}$ ). Significance of linear model was tested with ANOVA. Partial regression plots are shown in Fig. 3

associated with drought indices (Westerling et al. 2003). Yet drought progression also causes soil microbes to enter a dormant state which makes them more resistant to fire effects (e.g., Mataix-Solera et al. 2009 and citations therein).

Fire effects on soil chemistry are also likely to depend on fire intensity (Raison 1979). Fires often increase short-term availability of residual soil N (e.g., Vitousek and Howarth 1991; Wan et al. 2001) and P (e.g., Cui et al. 2010; Hartshorn et al. 2009; Raison 1979; Schaller et al. 2014). Yet intense fires will more fully combust biomass and volatilize nutrients (Certini 2005; Hatten and Zabowski 2010), especially N (Raison 1979). Thus, varying fire intensities may produce a spectrum of effects on soil chemistry. We detected a positive effect of fire intensity on soil N, P, and Zn but no effect on eight other soil nutrients. Increases in fire intensity resulted in linear increases in P and Zn. In contrast, low intensity fires caused an increase in N which quickly plateaued. A challenge is to then interpret the importance of such soil nutrient pulses on plants and soil microbial biomass.

In comparison to studies that have tested effects of fire on 1) plant and/or soil nutrients or 2) soil microbes,

our study is unique because it tested the effect of fire intensity on plant biomass, microbial biomass, and soil nutrients. While the breadth of our study and emphasis on fire intensity effects is relatively novel, we acknowledge a limit of our study. Many plant and soil properties were correlated with spatial distance along a major axis of the study site. This indicates the study site likely contained an edaphic gradient that affected plant and microbial biomasses. Plant and subsurface soil microbial biomasses were greater (63 and 61 %, respectively) at the left versus right ends of the study site (Fig. 1c). This additional source of variability may have increased the probability of type II errors. To help address this, we repeated tests for biomass measures with generalized least squares (GLS) (in place of ordinary least squares [OLS]) regressions which account for spatial autocorrelation in model residuals (Dormann et al. 2007). These additional tests had no appreciable impact on our interpretations except that subsurface microbial biomass was found to have a marginally significant ( $\alpha \leq 0.10$ ;  $P_{GLS} = 0.09$  versus  $P_{OLS} = 0.12$ ) positive association with fire intensity. Furthermore, plant biomass changed from having a non-significant positive ( $P_{OLS} = 0.44$ ,

**Fig. 3** Partial regression plots showing the relationship (slope and variance) between peak annual plant biomass (ANPP) and each variable while controlling for the influence of all other model

variables. Adjusted relationship between ANPP and boron (a) and phosphorus (b). Model details are shown in Table 3

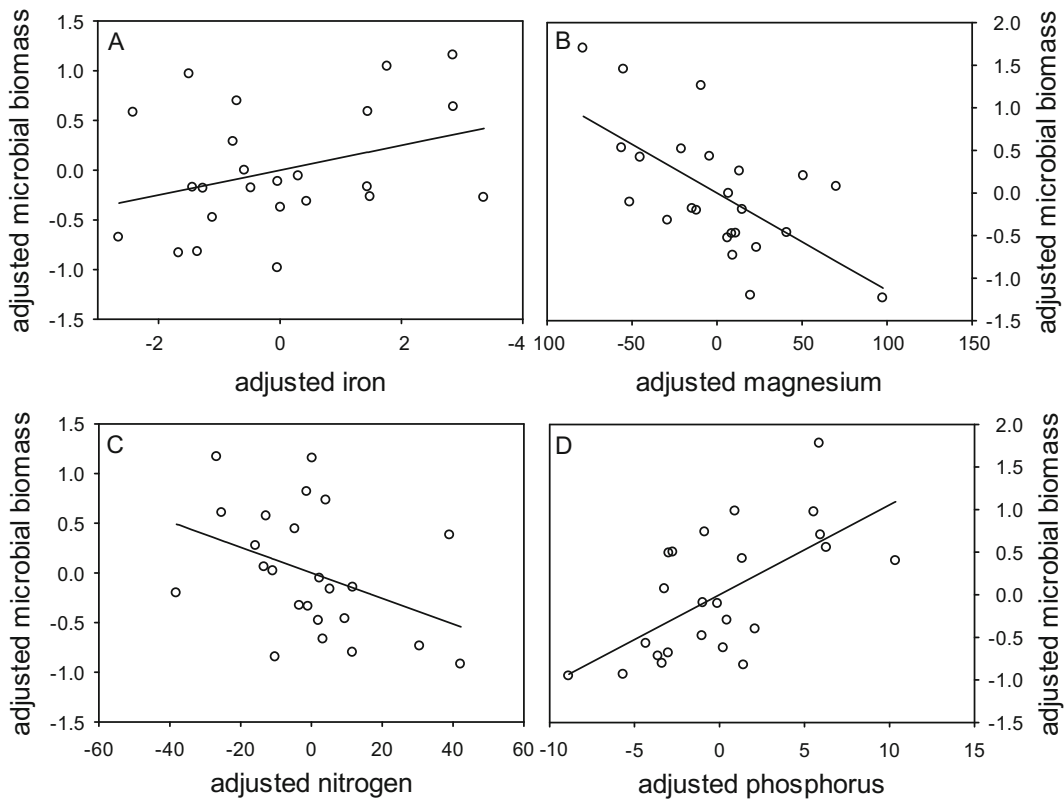
**Table 4** Best multiple regression model, based on Schwarz Bayesian Criterion scores, to explain variation in total microbial biomarker biomass (MB) near the soil surface (0–5 cm) and subsurface (5–15 cm)

Dependent variables	Independent variables	Beta coefficients	t-value	F <sub>4,19</sub>	P	R <sup>2</sup>	RI
MB <sub>0-5</sub>	Fe	0.63	2.52	–	0.021	–	0.12
	Mg	–0.77	–3.77	–	0.001	–	0.47
	N	–0.65	–2.78	–	0.012	–	0.17
	P	0.54	2.87	–	0.003	–	0.24
	total	–	–	–	4.54	0.010	0.49
MB <sub>5-15</sub>	Fe	0.39	1.58	–	0.13	–	0.05
	Mg	–0.73	–3.59	–	0.002	–	0.45
	N	–0.43	–1.86	–	0.079	–	0.06
	P	0.66	3.54	–	0.002	–	0.44
	total	–	–	–	4.79	0.008	0.50

MB = total microbial biomass ( $\mu\text{g} \times \text{g}^{-1}$  soil) and RI = relative importance (%). Variables included the following soil nutrients: Fe = iron, Mg = magnesium, N = nitrogen (nitrate and ammonium), and P = phosphorus ( $\mu\text{g}$  of nutrient  $\times 10 \text{ cm}^{-2} \times \text{burial time}^{-1}$ ). Significance of linear models was tested with ANOVA. Partial regression plots for the best subsurface microbial biomass model (MB<sub>5-15</sub>) are shown in Fig. 4

Table 2) to non-significant negative ( $P_{GLS} = 0.40$ ) relationship with fire intensity. Relative to the range of fire intensity levels in our experiment, we cautiously

conclude that fire intensity increased some soil nutrients (B, N, and P) but did not appreciably affect plant and soil microbial biomasses.



**Fig. 4** Partial regression plots showing the relationship (slope and variance) between subsurface (5–15 cm) microbial biomass and each variable while controlling for the influence of all other model

variables. Adjusted relationship between microbial biomass and iron (a), magnesium (b), nitrogen (c), and phosphorus (d). Model details are shown in Table 4

## ii. Associational patterns

Our dataset's inherent variability, due to fire treatments and spatial variation, was amenable to quantifying ecological associations (e.g., plant-nutrient, microbe-nutrient) which were then used to interpret nutrient (co-)limitations and biotic interactions. A strength of this dataset is that it enables assessing the importance of a large number of soil nutrients with some varying by fire intensity (P, N, Zn) and spatially (P, B, K). Lastly, we caution the reader that these are associational patterns. Additional experiments are necessary to confirm a nutrient is limiting.

A common belief in ecology is that terrestrial ecosystems are mainly limited by N (e.g., Craine and Jackson 2009; Hooper and Johnson 1999; Vitousek and Howarth 1991). Preliminary data on green grass nutrient stoichiometry ( $N:P = 6.1 \pm 0.3$  [mean of three dominant species  $\pm 95\%$  confidence interval], Kurt O. Reinhart, *unpublished data*) indicated dominant grasses at our study site were N-limited (e.g., Güsewell 2004; Koerselman and Meuleman 1996; Zechmeister-Boltenstern et al. 2015). [But see Craine et al. (2008) which found the N:P threshold values are often meaningless for grasslands.] Instead, we predicted grassland annual productivity was P-limited because the focal grassland has a B horizon with calcium carbonates which may immobilize P (Ehrenfeld et al. 2005; Jones et al. 2013). Furthermore, P is known to limit productivity in several grasslands (e.g., Chaudhary et al. 2009; Craine et al. 2008; Donaldson et al. 1984; Fay et al. 2015; Niklaus and Körner 2004; Penning de Vries et al. 1980; Snyman 2002) and mature ecosystems on weathered soils (e.g., Chadwick et al. 1999; Vitousek 1984). We found that plant biomass was positively associated with P (and co-limited by B and Mn) thereby suggesting that P limited annual plant biomass. We failed to detect an association between plant biomass production and N suggesting that all levels of available N were adequate. One reason that we may have failed to detect an important role of N may have been due to the presence of Mn in the dataset which was highly correlated with N (Table 2). To control for this, we repeated our multiple regression analysis without Mn as a potential predictor and found that the best model also did not include N (results not shown). Plants may also have responded to N in ways that were not readily detectable (e.g., increased root biomass). Based on the available results, the most plausible interpretation is that aboveground

plant biomass was limited mainly by P and select micronutrients. Plants and soil biota may use diverse tactics to increase mobilization and uptake of P (Ehrenfeld et al. 2005). For example, colonization of roots by mycorrhizal fungi (Reinhart and Anacker 2014) may help grassland plants take up P (Smith et al. 2011).

Other important nutrients that seemingly limited plant biomass production included B and Mn. Boron like P varied with a main spatial axis but unlike P was not appreciably affected by fire intensity. Variation in Mn could not be explained by a main spatial axis or fire intensity. Though B and Mn are critical plant nutrients, their relative importance in temperate grasslands is largely unknown. Some relevant studies exist in agronomic literature, with bean production being limited by P and Mn (Abdel-Reheem et al. 1992) and B, P, and Zn limiting strawberry production (May and Pritts 1993). Further experiments are needed to validate the relative importance of B, Mn, and P in grasslands.

Since nutrient dynamics are known to be context specific (e.g., Dijkstra et al. 2012), our findings may not be generalizable to other seasons, years, or sites. However, the results are not atypical since many other grassland systems report plant biomass production was limited (or co-limited) by P (Chaudhary et al. 2009; Craine et al. 2008; Donaldson et al. 1984; Fay et al. 2015; Niklaus and Körner 2004; Penning de Vries et al. 1980; Snyman 2002). Additionally across many reclaimed oil pads in the Northern Great Plains, plant cover was positively associated with soil P [and negatively associated with soil salts and N] (Erin K. Espeland, *personal communication*). Another consideration is that our study quantifies plant and microbial biomasses that follow recent fluxes in soil nutrients (i.e., 2–11 months) while some fire effects require more time to manifest (e.g., 2nd growing season after fire treatments; Vermeire et al. 2011). Nutrient pulses that occur the first growing season after fire may not result in increased aboveground production until the second growing season after fire. Variation in microbial biomass is likely to be the opposite and highly responsive to recent conditions in the prior days or week(s).

Plant biomass was positively correlated with microbial biomass. Unlike plant biomass, we found no correlations between surface (0–5 cm) or subsurface (5–15 cm) microbial biomasses and soil nutrients (Table 2). We only detected microbe-nutrient associations with multiple linear regressions. These results, however, suggest that microbial biomasses were also

limited by P. Interestingly, microbial biomass at both depths was negatively associated with N. Based on the results, we interpret that microbial biomasses were limited mainly by P and possibly limited by Fe.

We suggest three alternative experimental design considerations that may improve detection of microbe-nutrient associations: 1) collect PLFAs several times while ion resins are inserted and compare soil nutrients flux to mean microbial biomass per plot; 2) reduce plot size or have a blocked field experiment, instead of a completely randomized design, to limit the confounding effect of spatial gradients; and 3) if elucidating associational relationships is the main objective then collect all data types in many relatively small areas (1 m<sup>2</sup>) per field plot and maintain these as independent samples (Reinhart and Rinella 2016). While smaller plots have advantages, they may not be representative of most wild or prescribed fires because smaller plots are often burned with backfires which have different fire properties than head fires (Raison 1979 and citations therein).

## Conclusion

Fire ecology has progressed to the point that it is now important to understand the role of fire intensity within ecosystems. In the studied grassland, fire intensity positively affected N, P, and Zn but had no appreciable impact on plant biomass, soil microbial biomass, and other soil nutrients. Plant biomass was positively associated with P, B, and Mn (but not N) suggesting that P pulsed by fire may compensate for some unmeasured direct negative effect(s) of fire on plant communities. Soil microbial biomass was unaffected by fire intensity and exhibited some evidence of P and micronutrient limitations. Future research needs to test for multiple-nutrient constraints on grassland productivity and not assume that N is the only (or main) nutrient limiting grassland production.

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