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## Soil type and moisture regime control microbial C and N mineralization in grassland soils more than atmospheric CO<sub>2</sub>-induced changes in litter quality

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### ABSTRACT

Global change-induced alterations in litter quality and soil moisture regime will likely impact grassland C and N dynamics, but how these changes interact with edaphic properties across the landscape is unclear. We measured the effects of litter quality, soil type, soil moisture level, and soil drying-rewetting frequency (D-RW) on microbial C and N mineralization of litter and soil organic matter (SOM) in a full-factorial, controlled incubation experiment. Four levels of litter quality (no litter; or litter from *Bouteloua curtipendula* grown under 280, 380, 500  $\mu\text{L L}^{-1}$  CO<sub>2</sub>) were surface-applied to three contrasting soils common to Blackland Prairie landscapes: an upland Mollisol, a lowland Vertisol, and a fluvial Alfisol. Different soil moisture regimes were tested by incubating soils at four moisture levels (air-dry, 25%, 35%, or 50% water-holding capacity, WHC) and by drying-rewetting soils 0, 1, 2, 4 or 8 times over the 112-d incubation period. Litter additions stimulated microbial activity, increasing total CO<sub>2</sub> production (i.e. C mineralized from litter + SOM decomposition) up to 17 $\times$  more than no-litter controls (average 3 $\times$ ) and decreasing net N mineralization up to  $-3\times$  less (average  $-0.5\times$ ) due to greater microbial N immobilization. Neither C nor N mineralization, however, was affected by litter quality. For all soils, litter decomposition increased with increasing WHC and D-RW frequency, but the average percent of total CO<sub>2</sub> derived from litter was a negative function of SOM content. Similarly, net N mineralization also was positively correlated with soil WHC and affected most strongly by soil type (Alfisol < Mollisol < Vertisol). Net N mineralization responses to D-RW events was also soil-specific, with Alfisol soils showing no response and Mollisol and Vertisol soils decreasing after 4 D-RW events. Our results suggest that predicted changes in rainfall patterns and its interactions with soil type across the landscape will control short-term C and N mineralization responses in grasslands to a greater extent than atmospheric CO<sub>2</sub>-induced changes in litter C:N ratio for this common species of prairie grass.

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### 1. Introduction

Global changes such as rising atmospheric CO<sub>2</sub> and altered precipitation regimes are expected to impact grassland productivity by directly or indirectly altering the availability of water, nutrients, and energy (i.e. carbon, C; Dukes et al., 2005; Soussana and Lüscher, 2007; Leakey et al., 2009). The interaction of these global changes with edaphic characteristics on soil microbial activity likely will affect whether grasslands sequester or release C. Little data, however, are available on more spatially-explicit responses to global changes, such as those driven by landscape variation in soils.

Small-scale spatial variation in soils caused by vegetation can significantly affect plant-soil responses to elevated atmospheric CO<sub>2</sub>. For example, microbially-mediated C and N processes in soil microsites under shrubs are accelerated under elevated atmospheric CO<sub>2</sub> compared to soils in plant-free interspace microsites in a desert rangeland, particularly when water is less limiting (Billings et al., 2004; Jin and Evans, 2007; Schaeffer et al., 2007; Jin et al., 2011). In more mesic rangelands and grasslands, plant growth responses are mediated largely by elevated CO<sub>2</sub>-induced increases in plant water-use efficiency and soil moisture availability (Morgan et al., 2004; Körner, 2006; Polley et al., 2008) and, in some cases, greater N mineralization (Dijkstra et al., 2007, 2010). Furthermore, in a Blackland prairie system, soil type strongly affects how plant productivity and grassland species composition respond to increased soil water availability and subsequent changes to soil N

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availability under elevated CO<sub>2</sub> (Polley et al., 2003, 2012a, 2012b; Fay et al., 2012).

Soil CO<sub>2</sub> effluxes are high in tallgrass prairies compared to other temperate terrestrial ecosystems because of their relatively high plant productivity, annual precipitation, and soil organic matter (SOM) content (Mielenick and Dugas, 2000). Changes in the amount and variability of precipitation, however, are likely to regulate soil CO<sub>2</sub> effluxes and their underlying drivers (Fay et al., 2011) and responses to elevated atmospheric CO<sub>2</sub> (Jastrow et al., 2000). For example, grassland soil CO<sub>2</sub> fluxes decreased with increased time interval between rainfall events (Fay et al., 2000; Harper et al., 2005), in part due to reduced soil moisture and plant productivity and expected C input to soils (Fay et al., 2008).

More than half of the CO<sub>2</sub> emitted by grassland soils is from microbial SOM decomposition (Wan and Luo, 2003; Millard et al., 2008). Litter inputs and litter decomposition, however, confer substantial variability to overall soil CO<sub>2</sub> fluxes and the transfer of plant C into SOM. Increases in litter inputs may lead to more C stored as SOM (Franck et al., 1997; Gorissen and Cotrufo, 2000; Jastrow et al., 2000), and changes in litter quality affect the partitioning of C into different SOM pools (Melillo et al., 1982; Cotrufo et al., 1998; Guo and Gifford, 2002). Increased C inputs, however, can stimulate SOM decomposition (i.e. priming effect) and limit grassland C storage potential (Kuzyakov et al., 2000; Xie et al., 2005; Niklaus and Falloou, 2006; Carney et al., 2007). Further, the relationship between litter C:N ratio, which typically increases in plants grown under elevated CO<sub>2</sub>, and litter decomposition rate is highly variable (Franck et al., 1997; Gorissen and Cotrufo, 2000; Norby et al., 2001). Changes in the quantity and/or quality of plant litter inputs into soil can result in either net mineralization or immobilization of N and feed back to affect litter decomposition (Torbert et al., 2000). Interactions between altered litter inputs and precipitation regime, therefore, could affect litter decomposition and grassland soil C storage.

The objectives of this study were to evaluate how global change-induced alterations in litter quality and soil moisture regime interact with edaphic characteristics to affect microbial C and N mineralization. Microbial C mineralization was quantified as the decomposition of organic C in litter and SOM to CO<sub>2</sub>. Microbial N mineralization, the conversion of organic N to inorganic N (i.e. ammonium + nitrate), was quantified as the net change in soil inorganic N over the incubation period. A positive net change indicates microbial mineralization of N, and a negative net change indicates microbial immobilization of N. We removed the confounding influences of root respiration, climatic variation in the

field, and the cumulative impact of CO<sub>2</sub> enrichment on soils by incubating plant litter grown under three CO<sub>2</sub> levels with soils that had no exposure to elevated atmospheric CO<sub>2</sub> (Paul et al., 2001; Collins et al., 2000). Because short-term C mineralization rapidly quantifies litter quality differences (Haney et al., 2001; Haney and Franzleubbers, 2009), controlled incubations were conducted using four litter treatments (no litter; or litter from *Bouteloua curtipendula* grown under 280, 380, 500 μL L<sup>-1</sup> CO<sub>2</sub>) on three contrasting soils common to Blackland Prairie landscapes (Alfisol, Mollisol, Vertisol). Total CO<sub>2</sub> production and net N mineralization were measured in soils incubated in a full-factorial experiment with four levels of soil moisture and five levels of drying–rewetting frequency to represent different precipitation regimes. In addition, water-extractable organic C and organic N concentrations in pre- and post-incubated soils were measured to evaluate whether changes in these potentially labile pools correlated with microbial C and N activities. We hypothesized that: (1) litter additions would stimulate microbial activities (decomposition, net N mineralization) in all soils; (2) differences in litter C:N ratio due to growth-CO<sub>2</sub> level would be reflected by differences in microbial activity; (3) C and N mineralization would increase with greater soil moisture; and (4) soil-specific properties would interact with soil moisture regime as the dominant factor controlling litter decomposition and net N mineralization.

## 2. Materials and methods

### 2.1. Soil and litter collection

Soils (0–15 cm) from three soil orders commonly found in the Blackland Prairie region of Texas were collected in Spring 2008. These soils represent a broad range of texture, N and C contents, and hydrologic properties for grasslands in the southern portion of the U.S. Central Plains. An Alfisol (Bastsil series), Mollisol (Austin series), and Vertisol (Houston Black series) were collected from U.S. Army Corp of Engineers Stillhouse Hollow Lake area (Belton, TX), the USDA-ARS's Grassland, Soil, and Water Research Laboratory (GSWRL; Temple, TX), and the GSWRL watershed network (Riesel, TX), respectively. Bastsil soils are loamy fine sands (fine-loamy, siliceous, active, thermic Udic Paleustalfs) formed of alluvial sediments and commonly found on stream terraces. Austin soils are silty clays (fine-silty, carbonatic, thermic Udorthentic Haplustolls), typical of erosional uplands. Houston Black soils are heavy shrink-swell clays (very-fine, smectitic, thermic Udic Haplusterts) that dominate lowland areas. Selected soil properties are shown in Table 1. Fresh

**Table 1**

Initial properties for soils (0–15 cm) and *Bouteloua curtipendula* litter used in laboratory incubations (mean ± se; n = 3). Different letters for each property indicate significant differences between soil series or between growth-CO<sub>2</sub> levels (P ≤ 0.05). Soil texture data from Fay et al. (2009).

Soil order	Unit	Alfisol	Mollisol	Vertisol
Soil series name	–	Bastsil	Austin	Houston Black
Sand	%	71.9 ± 0.9a	11.7 ± 0.4b	10.9 ± 0.6b
Silt	%	20.8 ± 0.5a	45.3 ± 0.7b	43.0 ± 0.6b
Clay	%	7.3 ± 0.4a	39.3 ± 1.0b	49.8 ± 1.4c
Total inorganic C	g C kg <sup>-1</sup>	0.3 ± 0.1a	68.2 ± 1.0b	64.6 ± 1.0c
Total soil organic C (SOC)	g C kg <sup>-1</sup>	4.1 ± 0.4a	17.8 ± 0.4b	21.8 ± 0.6c
Total N	g N kg <sup>-1</sup>	0.6 ± 0.1a	1.7 ± 0.1b	5.6 ± 0.1c
Water-extractable SOC	mg C kg <sup>-1</sup>	137 ± 62a	226 ± 24b	376 ± 38c
Water-extractable organic N (ON)	mg N kg <sup>-1</sup>	16.3 ± 0.2a	3.9 ± 0.3b	9.8 ± 0.3c
Water-extractable inorganic N (IN)	mg N kg <sup>-1</sup>	7.4 ± 0.4a	9.0 ± 0.5b	7.9 ± 0.3a
<i>B. curtipendula</i> growth-CO <sub>2</sub> level		280 μL L <sup>-1</sup>	380 μL L <sup>-1</sup>	500 μL L <sup>-1</sup>
Initial litter C	mg C g <sup>-1</sup>	377 ± 3a	397 ± 1b	384 ± 2c
Initial litter N	mg N g <sup>-1</sup>	5.6 ± 0.1a	7.3 ± 0.1b	18.9 ± 2.0c
Initial litter C:N	–	67.3 ± 0.3a	53.1 ± 2.0b	22.7 ± 1.9c
C added in 0.25 g dry litter	mg C	94	99	96
N added in 0.25 g dry litter	mg N	1.4	1.8	4.7

soils were passed through a 2 mm sieve, large organic particles and/or coarse roots removed by hand, and air-dried before use.

Litter from *B. curtipendula* (Michx.) Torr., a widely distributed mid-grass prairie species, was harvested from the USDA-ARS's Lysimeter CO<sub>2</sub> Gradient (LYCOG) experiment (Temple, TX USA; 31°05'N, 97°20'W; Polley et al., 2008; Fay et al., 2009). Plants growing in Alfisol monoliths at 280, 380, or 500 μL L<sup>-1</sup> CO<sub>2</sub> sections within LYCOG were harvested at the end of the 2007 growing season as standing dead plant material. Only Alfisol monoliths yielded sufficient grass litter for this experiment at all CO<sub>2</sub> levels. Aboveground biomass was clipped at 5 cm above the soil surface and dried to constant mass at 60 °C. For each growth-CO<sub>2</sub> level, dried litter was cut into 1-cm segments, homogenized, then surface-applied to soils prior to incubation.

## 2.2. Incubation design

Soils were incubated in triplicate over a 112-day incubation period (16 weeks) at 25 ± 1 °C in the dark using a full factorial design with 3 soil types, 4 litter treatments (no litter; low, ambient, and high growth-CO<sub>2</sub>), 4 soil moisture levels (air-dry, 25%, 35%, 50% mean daily water holding capacity, or WHC), and 5 dry-rewet frequencies (D-RW; 0, 1, 2, 4, 8 events). Soil moisture treatments were selected to represent a broad range of current and potential precipitation regimes, including prolonged drought (i.e. constant air-dry) to frequent abundant precipitation (i.e. 50% WHC up to 8 D-RW events). In addition, the standard 25 °C incubation temperature used here closely approximates surface soil temperatures experienced in this region during the growing season (i.e. 4-year average of monthly soil T at 5 cm depth was ~26.5 °C from March through October; Neitsch et al., 2011). The 16-week incubation period was similar to those used in other soil and litter incubation studies (Fierer and Schimel, 2002; Gordon et al., 2008; Marhan et al., 2008; Rasmussen et al., 2008).

Soils in the air-dry treatment were maintained at that level throughout the incubation (i.e. D-RW frequency of 0 only), resulting in a total of 576 soil beakers incubated (192 beakers per soil type). For each incubation sample, 40 g of dry soil was added to a 50-mL polypropylene disposable beaker. Dry litter (0.25 g) was surface-applied to soils (i.e. no incorporation) to better represent field inputs of aboveground litter to soil. Corresponding rates of litter C and N applied per treatment level are shown in Table 1. Each soil beaker was placed in a gas-tight 1-quart canning jar containing a 10 mL alkali trap (1.0 M KOH) to absorb CO<sub>2</sub> and sufficient water to maintain humidity. Alkali traps were replaced on the third day after each re-wetting treatment, then every 7 or 10 days until the next D-RW event throughout the incubation period. Jar-blank traps were also collected in triplicate at each time point from non-soil incubation jars that contained all materials except soils (i.e. trap, water, empty beaker) to provide blank corrections for CO<sub>2</sub> trapped from air inside the incubation jar itself.

Total CO<sub>2</sub> production ( $C_{\text{total}}$ ; mg CO<sub>2</sub>-C kg<sup>-1</sup> dry soil) from mineralization of soil organic matter ( $C_{\text{SOM}}$ ) and litter ( $C_{\text{litter}}$ ) was determined by adding 2 M BaCl<sub>2</sub> to alkali traps and titrating to neutral pH with 1.0 M HCl (Anderson, 1982). All CO<sub>2</sub> values from soil incubation jars were first blank-corrected using non-soil incubation values. Litter-derived CO<sub>2</sub> was then calculated as the difference between litter-amended soils and no-litter controls (i.e.  $C_{\text{litter}} = C_{\text{total}} - C_{\text{SOM}}$ ). We assumed that litter addition had no priming effect on SOM decomposition.

Because soil textures differed considerably, soil moisture levels were standardized across all soil types using water holding capacity (WHC, %). 100% WHC was determined for each soil type as the gravimetric water content of soil that was saturated and allowed to drain freely over 6 h in a filter funnel. Air-dry treatments received

no water additions. Moist soil treatments were maintained at an average daily level of 25%, 35%, or 50% WHC over the incubation period. Water holding capacity treatment level was maintained by adding deionized water to soils whenever alkali traps were replaced. Calculation of average daily %WHC included days when soils were undergoing drying-rewetting (D-RW) treatments.

Soils exposed to D-RW treatments underwent 0, 1, 2, 4, or 8 drying events spaced evenly over the 112-day incubation period. Each dry-down event lasted 7 days (i.e. 1 D-RW event = 7 days drying; 2 D-RW events = 14 days drying; 4 D-RW events = 28 days drying; and 8 D-RW events = 56 days drying). Because soils exposed to more D-RW events experienced a greater number of days below the target daily %WHC level, these soils were rewetted to a slightly higher WHC level after each dry-down event to maintain the target daily average %WHC over the 112-day incubation period (*sensu* Fierer and Schimel, 2002) (Fig. 1). For each drying event, jar lids were removed to allow air-flow in the incubator to dry the soil. By day 7 of the dry-down period, soil %WHC approximated air-dry %WHC. The CO<sub>2</sub> produced during the total dry-down period was estimated by measuring one-day CO<sub>2</sub> at days 2, 4, 7 and linearly interpolating daily CO<sub>2</sub> production for intervening days. The CO<sub>2</sub> produced during the total dry-down period was then calculated by summing daily CO<sub>2</sub> production over the 7 days.

## 2.3. Soil and plant analyses

Soil concentrations of total organic C and N, water-extractable organic C and N, and water-extractable inorganic N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>2</sub><sup>-</sup>-N + NO<sub>3</sub><sup>-</sup>-N) were measured in pre- and post-incubated soils. Total soil organic C (SOC), soil total N (TN), and litter C and N was determined by dry combustion at 600 °C, and total soil C was measured by combustion at 900 °C (Vario Max CHN, Elementar, Hanau, Germany) (Fay et al., 2009). Soil inorganic C was calculated as the difference between total C and SOC.

Soils were extracted with deionized water (1:10), shaken for 1 h, centrifuged at 2500 rpm for 15 min, then the supernatant filtered through Whatman #1 filter paper. Dissolved inorganic C was removed from a 25 mL aliquot of each filtered extract by acidifying with 1 N HCl prior to measuring total dissolved organic C and total dissolved N (Apollo 9000, Teledyne Tekmar, Mason, Ohio).

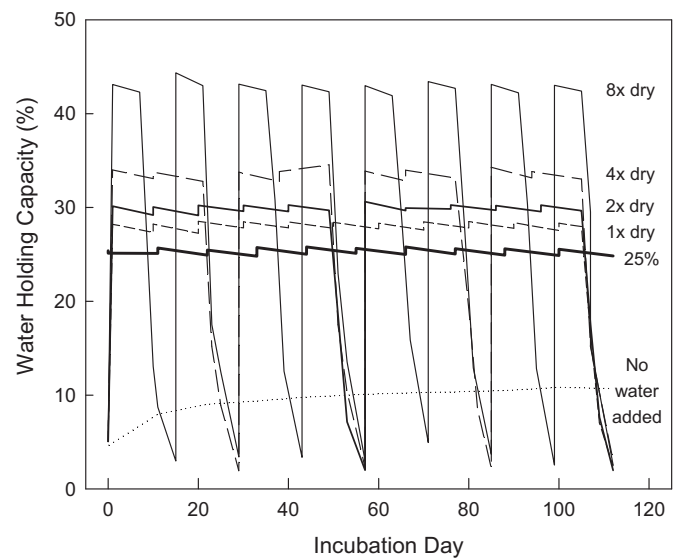


Fig. 1. Water holding capacity (WHC, %) for all soils and all drying treatments at the mean daily 25% WHC level over the 112-day incubation period. Values for the mean daily 35% and 50% WHC treatments followed similar patterns (data not shown).

Inorganic N was measured in non-acidified filtered extracts using continuous flow colorimetry (Flow Solution IV, OI Analytical, College Station, Texas). Organic N concentrations were calculated as the difference between total N and inorganic N. Net N mineralization ( $\text{mg N kg}^{-1}$ ) was calculated as the difference in inorganic N between pre- and post-incubated soils.

#### 2.4. Statistical analyses

Variables measured on pre-incubated soils and litter were analyzed using one-way analyses of variance (ANOVAs; PROC GLM) to test for differences in soil type and growth  $\text{CO}_2$  level, respectively. In post-incubated soils, four-way ANOVAs were used to test the fixed effects of soil type, litter, WHC, and D-RW treatments on  $C_{\text{Total}}$ ,  $C_{\text{litter}}$ , and on the relative contribution of litter mineralization (%) to total cumulative  $\text{CO}_2$  production. Final post-incubation soil C and N concentrations (total and water-extractable) and changes in water-extractable C and N concentrations (i.e. post-incubated – pre-incubated value) were also analyzed with four-way ANOVAs. Type III statistics are reported. Data were tested for normality using the Shapiro–Wilk statistic and transformed when necessary. Post-hoc multiple comparisons between significant treatment means were tested for differences using the Fisher's least significant difference (LSD) procedure. Linear regressions were used to examine correlations between cumulative  $\text{CO}_2$  production and net N mineralization vs. total and water-extractable C and N. All statistical tests were performed using SAS 9.1 (SAS, Inc., Cary, NC, USA).

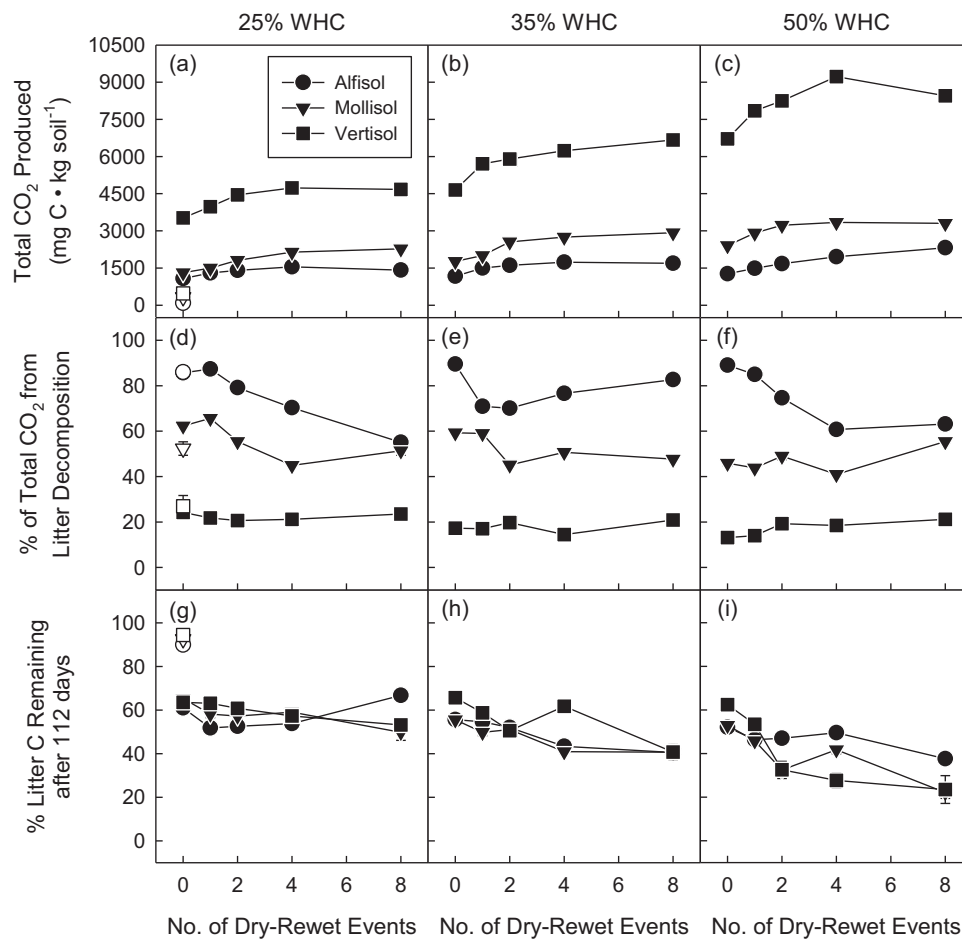
### 3. Results

#### 3.1. Pre-incubated soils and litter

Initial soil values for almost all physical and chemical properties differed by soil type ( $P \leq 0.05$ ; Table 1). Initial C and N concentrations of *B. curtipendula* litter differed by growth  $\text{CO}_2$  treatment level ( $P \leq 0.05$ ; Table 1). Although there were small but significant differences in litter C due to growth  $\text{CO}_2$ , differences in litter C:N ratio were driven by changes in litter N. Initial litter N values increased significantly with growth  $\text{CO}_2$  concentration. As a result, litter C:N ratios decreased from 67 to 23 with increasing growth  $\text{CO}_2$  concentration ( $P \leq 0.05$ ).

#### 3.2. Litter and SOM decomposition

Differences in litter C:N did not affect total C mineralization ( $C_{\text{Total}}$ ;  $\text{mg CO}_2\text{-C kg}^{-1}$  dry soil) from any soil under any soil moisture regime (WHC, D-RW) over the 112-day incubation. As a result, data were pooled across all litter additions, and results were re-analyzed using only two litter treatment levels: no litter ( $n = 3$ ) vs. litter added ( $n = 9$ ). Total  $\text{CO}_2$  production was affected by all treatment interactions ( $F_{16,480} = 2.40$ ;  $P_{\text{Soil} \times \text{WHC} \times \text{D-RW} \times \text{Litter}} = 0.002$ ) (Fig. 2), but variability in total  $\text{CO}_2$  production was dominated by soil ( $F_{2,480} = 15,033.2$ ), litter ( $F_{1,480} = 2786.63$ ), WHC ( $F_{2,480} = 2702.06$ ), the two-way interaction between soil and WHC ( $F_{4,480} = 1070.60$ ), and D-RW ( $F_{4,480} = 369.29$ ) (all  $P < 0.0001$ ). Total



**Fig. 2.** Carbon mineralized from soils and litter as: (a–c) total  $\text{CO}_2$  produced ( $\text{mg C} \cdot \text{kg soil}^{-1}$ ) from soil + litter,  $C_{\text{Total}}$ ; (d–f) the percent of total  $\text{CO}_2$  produced from litter ( $C_{\text{litter}}/C_{\text{Total}} \times 100$ ); and (g–i) the percent of litter remaining after 112 days. Litter addition treatments were averaged because growth  $\text{CO}_2$  treatment was not significant ( $n = 9$  per point). Standard errors are present but obscured by symbols when variability is low. Open symbols in 25% WHC panels indicate air-dried soil values.

CO<sub>2</sub> production was highest in finer-textured Vertisol soils, and lowest in coarser-textured Alfisol soils, and soil differences became more pronounced as %WHC increased (Fig. 2a–c).

Litter additions significantly increased total CO<sub>2</sub> production in all soils up to 17× (average 3×) more than no-litter controls at the same WHC and dry-rewet frequency (controls not shown). Litter decomposition dominated total CO<sub>2</sub> production in the coarse-textured, low organic matter Alfisol soils (54%–90% of total C mineralized, average 77%; Fig. 2d–f). In Mollisol soils, litter decomposition contributed to approximately half of total CO<sub>2</sub> production (41%–66%; average 52%). In the fine-textured, high organic matter Vertisol soils, total CO<sub>2</sub> produced was dominated by SOM decomposition, with litter decomposition contributing a minor but consistent percentage (13%–27%; average 20%). The total amount of litter decomposed increased (i.e. lower % litter C remaining; Fig. 2g–i) in all soils over the 112-day incubation as both %WHC and D-RW frequency increased, with significantly more litter decomposed in the finer-textured Mollisol and Vertisol compared to the Alfisol at 50% WHC.

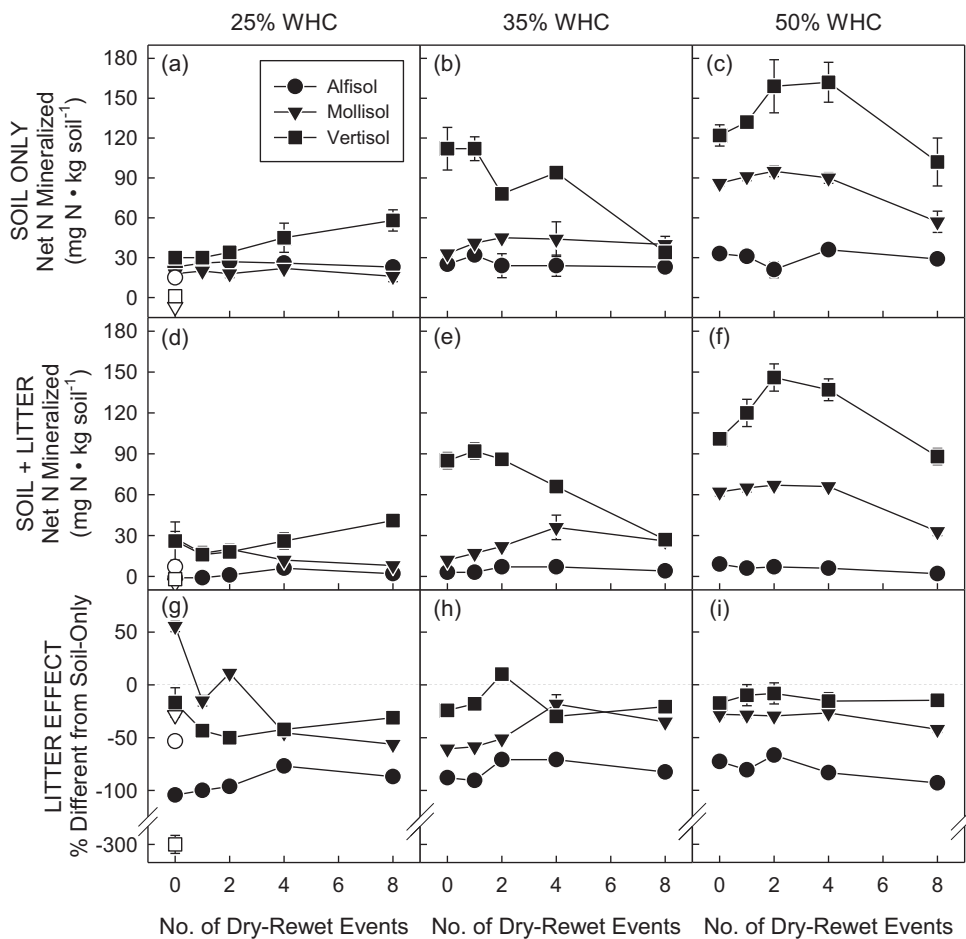
### 3.3. Net N mineralization

Similar to total CO<sub>2</sub> production, differences in litter C:N did not affect net N mineralization for any soil or soil moisture regime over the 112-day incubation, so the litter C:N levels data were pooled re-analyzed using only two levels, no litter vs. litter added. Net N

mineralization was affected by two-way interactions soil and litter ( $F_{2,480} = 3.18$ ;  $P_{\text{Soil} \times \text{Litter}} = 0.043$ ), WHC and litter ( $F_{3,480} = 4.59$ ;  $P_{\text{WHC} \times \text{Litter}} = 0.004$ ), and the three way interaction between soil, WHC, and D-RW ( $F_{16,480} = 9.72$ ;  $P_{\text{Soil} \times \text{WHC} \times \text{D-RW}} < 0.0001$ ). Net N mineralization was most strongly affected by soil type ( $F_{2,480} = 488.35$ ), WHC ( $F_{3,480} = 372.25$ ), the soil × WHC interaction ( $F_{4,480} = 130.68$ ), litter ( $F_{1,480} = 125.76$ ), and D-RW ( $F_{4,480} = 19.22$ ) (all  $P < 0.0001$ ).

Net N mineralization generally was lowest in the Alfisol and highest in the Vertisol, except at 25% WHC where net N mineralization was lowest in the Mollisol. Soil differences in net N mineralization grew more pronounced as %WHC increased (Fig. 3a–f). Adding litter decreased net N mineralization in all soils up to –3× (average –0.5×), indicating enhanced microbial N immobilization (Fig. 3g–i). Greatest levels of N immobilization occurred in the coarser-textured Alfisol compared to finer-textured Mollisol and Vertisol soils.

Net N mineralization in all soils averaged across all D-RW levels increased with %WHC (Fig. 3). The responses to D-RW frequency differed among soil type and WHC level (Fig. 3). Net N mineralization in the Alfisol were consistently low and did not respond to D-RW frequency at any WHC level, but both finer-textured soils showed D-RW frequency effects. In Mollisol soils, net N mineralization did not respond to D-RW frequency in no-litter controls, but decreased with D-RW frequency when soils were amended with litter at 25% WHC. In both no-litter and litter-amended Mollisol



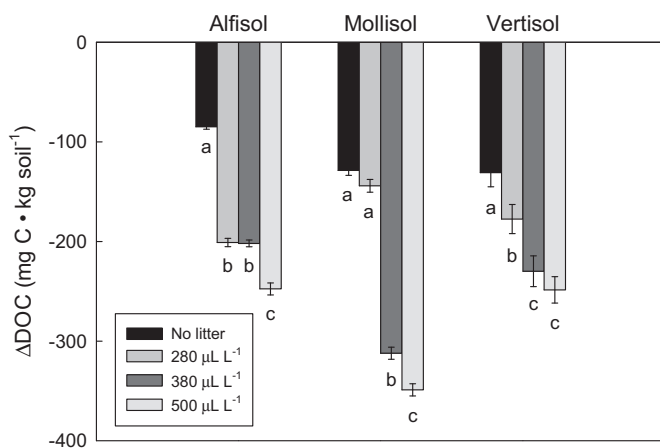
**Fig. 3.** Net N mineralized (mg N · kg soil<sup>-1</sup>) over the 112-day incubation in: (a–c) soils only; (d–f) soil + litter; and (g–i) due to litter only. Litter addition treatments were averaged because growth CO<sub>2</sub> treatment was not significant ( $n = 9$  per point). Values below dotted lines indicate net N immobilization. Standard errors are present but obscured by symbols when variability is low. Open symbols in 25% WHC panels indicate air-dried soil values.

soils at 35% WHC, net N mineralization increased up to 2–4 D-RW events then stabilized, but dropped after 4 D-RW events at 55% WHC. In Vertisol soils, net N mineralization increased with D-RW frequency at 25% WHC, decreased with D-RW frequency at 35% WHC, and decreased after 2–4 D-RW events at 55% WHC.

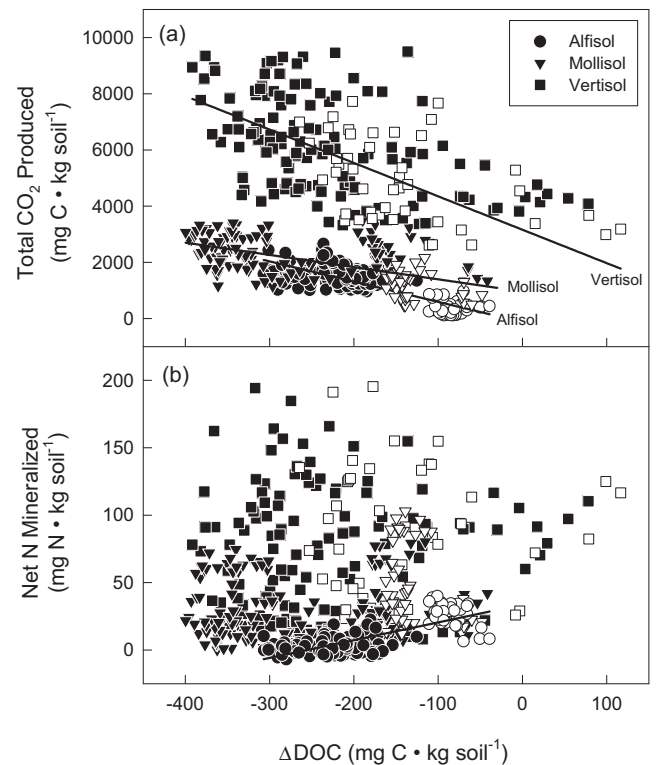
### 3.4. Changes in water-extractable DOC

Changes in water-extractable DOC ( $\Delta\text{DOC}$ ; post-incubated – pre-incubated concentrations) were calculated to evaluate whether decomposition of this potentially labile C pool was correlated with measured C and N mineralization. A positive  $\Delta\text{DOC}$  value indicates a net gain in DOC over the 112-day incubation, whereas a negative value indicates a net loss of DOC. Changes in water-extractable DOC were affected by the three-way interaction between soil, WHC, and D-RW ( $F_{6,555} = 4.27$ ;  $P_{\text{Soil} \times \text{WHC} \times \text{D-RW}} < 0.0001$ ) and the two-way interaction between soil and litter growth- $\text{CO}_2$  level ( $F_{6,555} = 43.03$ ;  $P_{\text{Soil} \times \text{Litter } \text{CO}_2} < 0.0001$ ). Mean  $\Delta\text{DOC}$  ( $\pm\text{se}$ ) were soil-specific but indicated DOC losses in all soils over the 112-day incubation. Litter additions stimulated greater losses of DOC compared to no-litter controls, with greatest losses occurring in the Alfisol ( $-149 \pm 10\%$ ) and the Mollisol ( $-131 \pm 21\%$ ), and lowest losses in the Vertisol ( $-24 \pm 16\%$ ). DOC losses were enhanced by increasing %WHC and D-RW frequency. Further, DOC losses were higher in all soils amended with litter grown at  $500 \mu\text{L L}^{-1}$  compared to 280 or  $380 \mu\text{L L}^{-1}$  (Fig. 4). Litter growth- $\text{CO}_2$  treatment differences in  $\Delta\text{DOC}$ , however, did not translate to differences in total  $\text{CO}_2$  production or net N mineralization in any soil at any WHC or D-RW level. Instead, all soils showed that DOC losses correlated with increases in total  $\text{CO}_2$  production (Alfisol:  $-7.2x - 128.9$ ,  $R^2 = 0.6127$ ,  $P < 0.0001$ ; Mollisol:  $-4.3x + 959.3$ ,  $R^2 = 0.2676$ ,  $P < 0.0001$ ; Vertisol:  $-11.9x + 3159.4$ ,  $R^2 = 0.4168$ ,  $P < 0.0001$ ; Fig. 5a). Only Alfisol soils also showed decreased N mineralization with greater DOC losses ( $0.13x + 33.6$ ,  $R^2 = 0.5738$ ,  $P < 0.0001$ ; Fig. 5b).

Changes in water-extractable organic N ( $\Delta\text{DON}$ ; post-incubated – pre-incubated concentrations) were not affected by litter additions or changes in WHC or D-RW level, and were not correlated to either total  $\text{CO}_2$  production or net N mineralization (data not shown). Averaged over all treatments, mean  $\Delta\text{DON}$  ( $\pm\text{se}$ ) was  $-12.3 \pm 0.4$ ,  $1.9 \pm 0.7$ , and  $4.4 \pm 1.3 \text{ mg N kg}^{-1}$  in Alfisol, Mollisol, and Vertisol soils, respectively.



**Fig. 4.** Change in water-soluble organic C concentration ( $\Delta\text{DOC}$ , post-incubated – pre-incubated soil values;  $\text{mg C} \cdot \text{kg soil}^{-1}$ ). Values are averaged over all WHC and D-RW treatments for each soil ( $\pm\text{se}$ ). Different letters within each soil type indicate significant differences in litter treatments ( $P \leq 0.05$ ).



**Fig. 5.** Relationship between water-soluble organic C concentration ( $\Delta\text{DOC}$ , post-incubated – pre-incubated soil values;  $\text{mg C} \cdot \text{kg soil}^{-1}$ ) and: (a) total  $\text{CO}_2$  produced ( $\text{mg C} \cdot \text{kg soil}^{-1}$ ; Alfisol:  $-7.2x - 128.9$ ,  $R^2 = 0.6127$ ,  $P < 0.0001$ ; Mollisol:  $-4.3x + 959.3$ ,  $R^2 = 0.2676$ ,  $P < 0.0001$ ; Vertisol:  $-11.9x + 3159.4$ ,  $R^2 = 0.4168$ ,  $P < 0.0001$ ); and (b) net N mineralized ( $\text{mg N} \cdot \text{kg soil}^{-1}$ ; Alfisol:  $0.13x + 33.6$ ,  $R^2 = 0.5738$ ,  $P < 0.0001$ ). Open symbols are no-litter control soils, closed symbols are litter-amended soils.

## 4. Discussion

Understanding the local mechanisms controlling soil–plant interactions and potential ecosystem feedbacks is critical for predicting landscape-level impacts of global change drivers. In this study, edaphic properties and average soil moisture exerted greater control than variability in soil moisture or litter quality on C and N mineralization in these temperate grassland soils. Litter additions increased total  $\text{CO}_2$  production in all soils compared to no-litter controls, but decreased net N mineralized by presumably increasing microbial N immobilization, partially supporting our first hypothesis. Net changes in water-extractable soil organic C (i.e.  $\Delta\text{DOC}$ ) over the 112-d incubation period indicated the greatest DOC losses (i.e. consumption or immobilization of DOC) in all soils amended with litter grown under elevated  $\text{CO}_2$ , but this did not translate to growth  $\text{CO}_2$ -specific changes in C or N mineralization, lending no support to our second hypothesis. Instead, increasing losses in DOC were broadly correlated with greater total  $\text{CO}_2$  production in all soils and decreased N mineralization in Alfisol soils. Overall, soil type and soil water availability far outweighed the minor effects of drying-rewetting (D-RW) frequency, and soil-specific interactions controlled microbial activities, supporting our third and fourth hypotheses.

### 4.1. Effects of growth $\text{CO}_2$ level on litter quality

Litter C:N ratios typically are higher in plants grown at elevated than ambient  $\text{CO}_2$  because N is diluted by the additional carbohydrates accumulated at high  $\text{CO}_2$  (Gorissen and Cotrufo, 2000;

Loladze, 2002; Taub and Wang, 2008). In contrast to this expectation, which also formed part of our first hypothesis, we found that litter C:N decreased with increasing growth CO<sub>2</sub>-concentration. A previous study from this site reported the expected positive correlation between C:N in plants harvested mid-season with growth CO<sub>2</sub> concentration (Gill et al., 2002), but this initial correlation did not persist after additional years of CO<sub>2</sub> treatment (Polley et al., 2003). Indeed, the most recent study from this site reported no significant CO<sub>2</sub> enrichment effects on the N concentration of grass tillers at mid-season on any of the three soil types (Polley et al., 2011). In this study, we measured C:N values in standing dead or senesced grass litter harvested at the end of the season. Litter C:N ratios at this time may have been influenced by N uptake late in the growing season and/or differences in end-of-season N translocation among CO<sub>2</sub> treatments. Specifically, CO<sub>2</sub> enrichment may have increased soil N availability to plants late in the season by reducing transpiration rates (Polley et al., 2008) and increasing soil water content (Jones and Donnelly, 2004; Dijkstra et al., 2008, 2010), resulting in greater litter N concentrations at higher CO<sub>2</sub>. CO<sub>2</sub> enrichment also has been shown to reduce translocation of N from aboveground biomass between mid- and late-season (Dijkstra et al., 2008). A similar mechanism may have contributed to the significant decrease in litter C:N at high CO<sub>2</sub> that we observed.

#### 4.2. Litter effects on C and N mineralization

Litter additions stimulated microbial activity, increasing total CO<sub>2</sub> production up to 17× more than no-litter controls (average 3×) and decreasing net N mineralization up to −0.5× less (average −0.5×) due to greater microbial N immobilization. Neither C nor N mineralization, however, was affected by differences in litter quality as observed by others (O'Neill and Norby, 1996; Cotrufo et al., 1998; Gorissen and Cotrufo, 2000; Torbert et al., 2000) but in contrast to other studies which found that total CO<sub>2</sub> production decreases with increasing litter C:N (Melillo et al., 1982; Moretto et al., 2001; Norby et al., 2001; Marhan et al., 2008). One potential source of error, which was not quantified here due to methodological constraints, is the role of soil-specific priming on respired CO<sub>2</sub>. Although we were not able to quantitatively partition respired CO<sub>2</sub> into component sources of litter decomposition vs. soil-specific priming, multiple studies have shown that litter-associated soil priming can enhance SOM mineralization and limit soil C sequestration potential (Kuzakov et al., 2000; Xie et al., 2005; Niklaus and Falloon, 2006; Carney et al., 2007).

Differences in litter C:N also did not affect water-soluble soil organic C or organic N concentrations (Marhan et al., 2008) in pre-incubated or post-incubated soils. Litter C:N, however, did affect the change in water-soluble, or dissolved, organic C ( $\Delta$ DOC; post-incubated concentrations – pre-incubated concentrations). A negative  $\Delta$ DOC value suggests that more labile C was immobilized or consumed over the incubation period and potentially released as CO<sub>2</sub>. The absence of litter C:N differences due to growth-CO<sub>2</sub> treatment effects on microbial activity suggests that the additional DOC in soils amended with litter grown under elevated CO<sub>2</sub> may have been immobilized in microbial biomass, transformed to a less bioavailable form of organic C, or sorbed to the soil matrix. Furthermore, changes in DOC concentrations were a magnitude smaller than soil respiration, so changes in  $\Delta$ DOC due to litter quality may have been too low to detect as respired C. Although these litter C:N differences may have been expressed after a longer incubation period, shorter-term C mineralization and the formation/stabilization of SOC will likely be controlled by litter quantity and its interaction with the soil matrix more than litter quality (Liu et al., 2009; Gentile et al., 2011).

#### 4.3. Soil type effects

Relationships between C mineralization and distinct soil properties such as mineralogy (Percival et al., 2000; Rasmussen et al., 2008), soil microsite (Billings et al., 2004; Schaeffer et al., 2007), and soil aggregate fractions (Williams et al., 2000; Gentile et al., 2011) suggest that variation in soil properties linked to landscape position will influence landscape-level ecosystem responses to global changes. In this study, soil type was a dominant factor affecting both litter and SOM decomposition in temperate grassland soils. In addition, litter additions interacted with soil type to affect total CO<sub>2</sub> production over the 112-day incubation period. In general, the C mineralized from litter decomposition contributed far less to the total CO<sub>2</sub> production in the fine-textured soils (Mollisol, Vertisol) than in coarse-textured soils (Alfisol). Decomposition of native SOM dominated CO<sub>2</sub> production in the Vertisol and less so in the Mollisol, whereas litter decomposition was the primary C source for mineralization in the low organic, coarse-textured Alfisol. Increased C inputs from added litter also could have further stimulated SOM decomposition in the Mollisol and Vertisol soils compared to the Alfisol soil, but partitioning a priming effect was beyond the scope of this experiment. In these Blackland Prairie soils, soil type was the primary controller determining the relative contribution of litter-C to total soil C mineralized.

Soil-specific differences in N mineralization and immobilization were likely due to different sized soil N pools in each soil type, with the Vertisol soils having a significantly larger total N pool compared to the other two soil types. Further, this large native N pool in the Vertisol soils may have allowed greater net N mineralization to occur at low WHC levels, which limited litter decomposition and microbial N immobilization.

#### 4.4. Soil moisture effects

Average soil moisture also was a major factor controlling litter and SOM decomposition. In this study, total CO<sub>2</sub> production increased with increasing %WHC, and the %WHC effect became more pronounced as SOM content increased. Further, soil drying-rewetting enhanced CO<sub>2</sub> production compared to constantly moist soils, primarily because drying-rewetting stimulated SOC mineralization to a greater extent than litter decomposition in all soils. Similarly, net N mineralization increased with WHC, and was sensitive to D-RW frequency in the fine-textured Mollisol and Vertisol soils, but not in the coarse-textured Alfisol soils. Although the average annual soil CO<sub>2</sub> flux in tallgrass prairie soils increases with annual precipitation (Mielnick and Dugas, 2000), pulses in water and subsequent nutrient availability after rainfall trigger pulses in biological activity (Fierer and Schimel, 2002; Austin et al., 2004; Xiang et al., 2008). Some modeling studies estimate that pulse responses can account for up to 14% of annual CO<sub>2</sub> fluxes in some ecosystems (Yuste et al., 2005; Li et al., 2006). Other studies have also found increased cumulative CO<sub>2</sub> production from soils undergoing multiple drying-rewetting events compared to soils kept at constant optimum water content (Denef et al., 2001; Miller et al., 2005; Xiang et al., 2008), although some studies have shown decreases (Franzluebbers et al., 1994; Fierer and Schimel, 2002; Mikha et al., 2005). In one study, less frequent but larger rainfall events were linked to lower soil CO<sub>2</sub> emissions because substrate limitation exerted greater control on soil CO<sub>2</sub> efflux relative to water or temperature under this more extreme rainfall pattern (Harper et al., 2005).

When soils remained wetter for longer (i.e. decreased D-RW frequency), the contribution of litter-C to total CO<sub>2</sub> produced increased. This is consistent with a field study showing that lower soil moisture status due to reduced precipitation treatment in



tallgrass prairie can significantly reduce litter decomposition (Reed et al., 2009). Because these negative effects can persist even after precipitation inputs are returned to normal (Reed et al., 2009), interannual variability in soil moisture has both short- and long-term effects on soil C mineralization (Fierer and Schimel, 2002; Harper et al., 2005).

## 5. Conclusions

Nutrient and energy flows in grassland ecosystems under increasing atmospheric CO<sub>2</sub> ultimately will depend on how microbially-mediated soil processes respond to variability in current and future rainfall patterns (Weltzin et al., 2003; Mikha et al., 2005; Schimel et al., 2007; Gordon et al., 2008). Results from this study imply that grassland C mineralization will be controlled more in the short-term by changes in the amount of precipitation and its interactions with soil type than by CO<sub>2</sub>-caused changes in litter C:N ratio. Thus, scaling-up controlled incubation results to complex landscape-level responses will be constrained by how landscape variation in soils interacts with this and other grassland species to affect how C and N cycling in grassland ecosystems will respond to altered precipitation patterns and increasing atmospheric CO<sub>2</sub> (Polley et al., 2003; Dijkstra et al., 2010; Fay et al., 2011, 2012; Polley et al., 2012a, 2012b).

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