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Changes in southern Piedmont grassland community structure and nutritive quality with future climate scenarios of elevated tropospheric ozone and altered rainfall patterns

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ABSTRACT

Forage species common to the southern USA Piedmont region, *Lolium arundinacea*, *Paspalum dilatatum*, *Cynodon dactylon* and *Trifolium repens*, were established in a model pasture system to test the future climate change scenario of increasing ozone exposure in combination with varying rainfall amounts on community structure and nutritive quality. Forages were exposed to two levels of ozone [ambient (non-filtered; NF) and twice ambient (2×) concentrations] with three levels of precipitation (average or ±20% of average) in modified open-top chambers (OTCs) from June to September 2009. Dry matter (DM) yield did not differ over the growing season between forage types, except in primary growth grasses where DM yield was higher in 2× than NF treatment. Primary growth clover decreased in nutritive quality in 2× ozone because of increased concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Re-growth clover exhibited the largest decrease in nutritive quality, whereas grasses were not adversely affected in 2× ozone. Re-growth grasses responded positively to 2× ozone exposure, as indicated in increased relative food value (RFV) and percentage crude protein (CP) than NF-exposed re-growth grasses. Effects of precipitation were not significant over the growing season for primary or re-growth forage, except in primary growth grasses where DM yield was higher in chambers with above average (+20%) precipitation. Total canopy cover was significantly higher over the growing season in chambers receiving above average precipitation, but no significant effects were observed with ozone. Results indicate shifts in plant community structure and functioning related to mammalian herbivore herbivory in future climate change scenarios.

INTRODUCTION

Tropospheric (ground level) ozone is a phytotoxic air pollutant that is globally pervasive and can be transported from metropolitan areas to rural, agricultural and forested lands (Chameides *et al.* 1994; Ashmore 2005). Middleton (1956) first reported ozone as phytotoxic, and since then the US EPA (2006, 2013) has recognised it as the most significant air pollutant affecting vegetation in the USA. The southern USA has a warm climate and dense vegetative cover that is a source of organic hydrocarbons, both of which contribute to the production of tropospheric ozone (Chameides *et al.* 1988; National Research Council 2004).

The Earth's climate has been altered dramatically over the last century and is projected to continue to change (Christensen *et al.* 2007; US Climate Change Science Program 2008; IPCC 2013). Some models predict that tropospheric ozone concentrations will increase globally by *ca.* 20% (0.3–2.0% year^{-1}) over the next 20 years (Thompson 1992; Vingarzan 2004). Also, summer rainfall may decrease or increase based on model predictions for the southeast USA (MacCracken *et al.* 2001). The Canadian and Hadley global circulation models (GCM) predict various increases in temperatures and total annual rainfall amounts for the southeast USA, although the Hadley model estimates 20% more rainfall in the summer months whereas the Canadian model predicts 10% less precipitation (MacCracken *et al.* 2001). Increasing ozone concentrations in combination with other factors involved in climate change, such as elevated temperatures, increased nitrogen deposition and altered precipitation events, will potentially cause perturbations in species composition and function in grassland communities (Ashmore & Ainsworth 1995; Power & Ashmore 2002; Bassin *et al.* 2007; Ren *et al.* 2007; Suttle *et al.* 2007).

Long-term exposure to elevated ozone concentrations over an entire growing season can lead to decreased plant growth and productivity (Barbo *et al.* 1998; Muntifering *et al.* 2006; US EPA 2006, 2013). In addition, ozone-induced changes in foliar chemistry may result in decreased nutritive quality of herbaceous vegetation for ruminants (Krupa *et al.* 2004) and other mammalian herbivores (Gilliland *et al.* 2012).

Barbo *et al.* (1998) showed that ozone could shift community richness and evenness in an old-field community, as well as increase visible foliar injury in blackberry (*Rubus cuneifolius*). Vegetation exposed to CF (carbon-filtered) air had higher species richness and diversity, and the plant community was more vertically dense than plants exposed to ambient and twice ambient (2×) ozone concentrations. Increasing ozone concentrations have detrimental effects on sensitive plant species in grasslands (Ashmore & Ainsworth 1995; Power & Ashmore 2002; Bender *et al.* 2006; Hayes *et al.* 2007), and can alter community structure and function (Fuhrer *et al.* 1994; Barbo *et al.* 1998; Kim *et al.* 1998; Gonzalez-Fernandez *et al.* 2008). Indirect effects, or community-level responses, to ozone in cool-season grasses have been reported in Europe (Ashmore *et al.* 1995; Power & Ashmore 2002; Hayes *et al.* 2009). In a study in the southeast USA to determine ozone effects on cool-season clover–fescue pastures, Heagle *et al.* (1989b) reported that growth of white clover (*Trifolium repens*) was reduced after ozone and water stress, whereas growth of tall fescue (*Lolium arundinacea*) increased. Hayes *et al.* (2009) examined inter- and intra-species competition of *T. repens* (clover) and *L. perenne* (grass) using mesocosms in solar domes in the UK. The authors reported that the clover was more sensitive than the grass species, and the sensitivity was directly related to a decrease in photosynthetic capacity. Using mesocoms, Gonzalez-Fernandez *et al.* (2008) reported white clover nutritive quality and canopy cover decreased after ozone treatment, whereas ryegrass (*Lolium multiflorum*) permeated areas previously occupied by the clover when the two were grown together. It is important that whole plant communities be investigated and the effects of ozone exposure are quantified to gain understanding of inter-specific responses to ozone and competition (Heagle *et al.* 1989b, 1991; Barbo *et al.* 1998, 2002; Davison & Barnes 1998; Lewis *et al.* 2006; Gonzalez-Fernandez *et al.* 2008).

The main hypothesis of our study was that in future climate change scenarios, elevated tropospheric ozone concentrations alone or in combination with altered rainfall amounts would adversely affect resource allocation, canopy structure and nutritive quality of dominant forage species found in the southern Piedmont region of the USA. This region, which covers 17 million ha from central Virginia to eastern Alabama (USDA 1981), is experiencing rapid population growth (Alig *et al.* 2004) and includes a number of areas that are not compliant with current NAAQS for ozone (US EPA 2013). Our specific objectives were: (i) to determine if response to ozone varies among grasses and/or a legume (clover) regarding community structure and function (nutritive quality); and (ii) to ascertain whether species composition, canopy structure and nutritive quality are affected by a combination of elevated ozone and predicted future precipitation regimes.

MATERIAL AND METHODS

Study site, ozone exposure and rainfall treatment

The study site was located *ca.* 5 km from Auburn University and was representative of non-intensively managed grasslands throughout the southern Piedmont region (US Department of Agriculture 1981). The ozone exposure system consisted of 12 large open-top chambers (OTC; 4.8-m high × 4.5-m diameter; Heagle *et al.* 1989a). Each OTC had a hood at the top to exclude ambient precipitation, but allow ambient air circulation (Manning & Krupa 1992). Using principal components analysis (Gomez & Gomez 1984), the chambers were selected on the basis of uniformity of initial plant communities and soil characteristics.

Each chamber was aerially seeded with a combination of three common grasses and one legume species found in the southern Piedmont region (Ball *et al.* 2002): tall fescue (*Lolium arundinacea*, C_3, cool season grass), dallisgrass (*Paspalum dilatatum*, C_4, warm season grass), ladino clover (*Trifolium repens*, C_3, cool season legume) and common bermudagrass (*Cynodon dactylon*, C_4, warm season grass). Prior to seeding, existing vegetation was mowed in each chamber and treated with glyphosate, then the soil was tilled, all dead vegetation removed, and the soil surface raked and seeded (*ca.* 1 week after herbicide treatment). Tall fescue and white clover were aerially seeded in October 2007 at rates of 7.00 and 0.84 kg pure live seed (PLS) ha^{-1}, respectively. Dallisgrass and common bermudagrass were seeded in March 2008 at rates of 4.20 and 2.80 kg $PLS{\cdot}ha^{-1}$, respectively. After seeding the area was covered with *ca.* 1 cm of soil and wheat straw and each chamber misted daily to enable germination. Seeding rates for all species were established on the basis of Alabama Cooperative Extension System recommendations (D. Ball, personal communication). Prior to the 2009 growing season (early March), all chamber vegetation was cut to 0.5 cm to ensure uniformity.

The two ozone treatments applied in this study were: non-filtered air (NF), representative of ambient air found in rural areas within the Piedmont region (US EPA 2006, 2013); and air enriched to twice ambient (2× ambient) ozone concentration, *i.e.* future ozone predictions for rural areas in the Southern Piedmont region (Lefohn *et al.* 1992; Thompson 1992; Vingarzan 2004). There were six replicates (OTCs) of both the NF and 2× ozone treatments.

Water lines were placed underground and routed to the centre of each chamber, *ca.* 0.5 m above ground level, for irrigation purposes. Individual radial sprinklers were attached to each water line, and their operation controlled by automatic timers and manipulated in zones of four chambers each (one zone per rain treatment). Operational constraints of the irrigation system allowed precipitation to be grouped into three zones (blocks) that were then used in the split-plot design with two ozone treatments per precipitation zone. An individual water meter was attached to each sprinkler to measure the amount of water dispensed per simulated rain event. Water was applied three times daily, recorded weekly, and converted to cm rainfall to ensure chambers received target precipitation amounts. Styrofoam cups were also randomly placed in each chamber to ensure uniform water distribution throughout the chambers. The 12 chambers were blocked into three zones of varying precipitation amount (four chambers per precipitation treatment). Each water treatment zone contained two chambers receiving NF air and two receiving 2× ambient ozone. The three different precipitation blocks were used to simulate future predicted rainfall amount: zone 1, average (30-year average monthly rainfall for Auburn, AL, USA); zone 2, average monthly rainfall +20% (Hadley GCM); and zone 3, 20% less than average (representative of Canadian GCM; MacCracken *et al.* 2001). These rainfall amounts were selected based on the uncertainty of future rainfall predictions for the southeast USA

(Burkett *et al.* 2001; MacCracken *et al.* 2001; Christensen *et al.* 2007) and on average monthly rainfall in the Auburn AL area from 1971 to 2000 (Alabama Weather Information System).

Ozone fumigation and rainfall treatments were initiated on 1 June and continued until 30 September. Ozone was generated by passing pure oxygen through a high-intensity electrical discharge source (Griffin Inc., Lodi, NJ, USA) and added proportionally above ambient (NF) to the chambers for 12 h·day^{-1} (09:00–21:00 h), 7 days·week^{-1}. Fans were turned off from 23:00 to 05:00 h to allow for natural dew formation within the chambers. Instruments were calibrated according to US EPA quality assurance guidelines.

Plant measurement

In each chamber two randomly selected permanent plots of 0.5-m diameter each were delineated with circular metal rings on the ground. These plots were harvested each month to determine re-growth capacity of each forage type (clover and grasses). In addition, primary growth forage from two randomly selected plots (0.5-m diameter) within each chamber was harvested each month. Plots within chambers were assigned randomly for each harvest and designated for selection using a random numbers table in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Primary and re-growth plots were established to investigate whether ozone adversely affects certain species at different stages of growth, and to simulate mechanical harvesting of pasture for hay production (Muntifering *et al.* 2000; Bender & Weigel 2003). Because seasonal distribution of forage growth varies among species (Ball *et al.* 2002), periodic harvests throughout the growing season were expected to contain an optimal representation of cool season C_3 grasses, warm season C_4 grasses and clover. Consideration was given to edge effects associated with OTCs (Fuhrer 1994) and accounted for by maintaining an effective exposure area of within 0.5 m from the inside edge of the chambers.

A point-frame canopy cover count was conducted before each harvest using a method modified from Bonham (1989) and Barbo *et al.* (1998). This device used was circular in design and covered an area of *ca.* 0.2 m^2 per plot (two plots each for the permanent and re-growth plots). Total percentage canopy and individual species cover was evaluated each month throughout the duration of the study. For purposes of this study, the percentage canopy cover was separated into grasses and clover, with all grass species from each plot pooled into a single 'grass' sample per chamber. The total effective exposure area in each chamber was 9.5 m^2, with *ca.* 10% of this area sampled each month.

Laboratory analysis

Samples (two each collected from the plots referenced above) from each monthly harvest of primary and re-growth forage were cut with scissors to 0.5 cm from ground level, placed in paper sacks and transported to the laboratory. The samples were then manually separated into grasses and clover, with all grass species from each plot pooled into a single 'grass' sample (one re-growth, one primary growth) per chamber. Clover dry matter (DM = biomass) in the average precipitation chambers was insufficient for laboratory analysis and consequently was omitted, leaving only comparisons of above and below average precipitation treatments in clover forage analysis. After separation of forage types, harvested material was dried in a conventional forced-air oven at 50 °C to constant weight, air-equilibrated and weighed for DM yield. Samples were then ground in a Wiley mill to pass through a 1-mm screen, and sequentially fractionated according to Van Soest *et al.* (1991) for isolation of cell wall constituents (hemicellulose, cellulose and lignin): neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). An ANKOM fibre analyser (ANKOM Technology Corp., Fairport, NY, USA) was used for sequential detergent fractionation of samples. Percentage DM and concentration of crude protein (CP; Kjeldahl N × 6.25) in samples was calculated according to the Association of Official Analytical Chemists (1995). Relative food value (RFV), an index of forage quality that integrates forage DM intake and digestibility (Rohweder *et al.* 1978), was calculated from forage concentrations of NDF and ADF using prediction equations of Linn & Martin (1989).

Experimental design and statistical analysis

The experimental design for this field fumigation study was a randomised split-plot design with two ozone treatments replicated within three zones (blocks) of precipitation, and forage re-growth and primary growth plots within each treatment. The experimental unit was the OTC, with plant communities in each OTC representing individual sampling populations. Overall effects are presented using *P*-values, with a rejection level of the null hypothesis set at $P = 0.10$ (Peterman 1990). Treatment effects were analysed using univariate ANOVA and simple *t*-tests were used to test significance within treatments for ozone. To test differences among primary and re-growth harvests over time multivariate ANOVA was used. Analyses were conducted using the R package for statistical analysis (R Development Core Team 2010).

RESULTS

Climate data and ozone exposure

Monthly air temperatures (24-h average) were similar to the 30-year averages throughout the growing season at 26.0 and 25.1 °C, respectively, for June and September 2009. Total precipitation in the Auburn area for June–September 2009 was 47.6 cm, compared with 44.0 cm for the 30-year average (ncdc.noaa.gov). Average simulated monthly rainfall amounts for the three rainfall regimes were 11.9, 15.5 and 9.7 cm for average, +20% (high) and -20% (low), respectively, close to target average monthly rainfall of 11.4 cm for average, 13.7 cm high ozone, 9.1 cm low ozone for each precipitation zone in each month (Table 1).

Mean 12-h (09:00–21:00 h) ozone concentrations over the 4-month experiment were 31 and 56 nl·l^{-1} (ppb), respectively, for NF and 2× treatments (Table 2). Average peak ozone concentrations were 39 and 77 nl·l^{-1} for NF and 2× treatments. Peak average 1-h ozone concentrations were 73 and 155 nl·l^{-1} for NF and 2× treatments, respectively. From the data, two cumulative exposure response metrics were developed: (i) AOT40 is the accumulated amount of ozone above the threshold value of 40 ppb, and (ii) A sigmoidal weighting function (W126) developed by Lefohn & Runeckles (1987). The total

Table 1. Monthly average target and actual precipitation values (cm) over the growing season for chambers by month and precipitation regime.

	average		high (+20%)		low (−20%)	
month	target	achieved	target	achieved	target	achieved
June	11.4	11.8	13.72	15.7	9.1	9.7
July	11.4	12.22	13.72	16.0	9.1	9.6
August	11.4	11.22	13.72	15.3	9.1	9.5
September	11.4	12.22	13.72	15.0	9.1	9.8
total	45.7	47.5	54.9	62.0	36.6	38.6
season monthly average	11.4	11.9	13.7	15.5	9.1	9.7

Average target = average precipitation in chambers 1–4, (30-year average from 1971 to 2000 for Auburn area); High = chambers 5–8, 20% more than average; Low = chambers 9–12, 20% less than average (Canadian GCM).

12 h AOT40 values for the two treatments throughout the 2009 growing season were 1.8 and 29.8 $ppm \cdot h^{-1}$ for NF and 2× treatments, respectively. Seasonal 12 h W126 values ($ppm \cdot h^{-1}$) were 1.6 and 42.5 for NF and 2× treatments, respectively (Table 2).

Plant measurements

Primary growth

Primary growth grasses, on average, increased growth in 2× ozone treatments over the growing season, with a 19% increase in DM yield in 2× treatments compared with NF-exposed grasses (Tables 3 and 4). Clover decreased in nutritive quality in 2× ozone treatments, with significant ($P = 0.05$) increases in ADL (Table 3). In the final harvest, CP concentration was 6% higher ($P = 0.10$) in 2× ozone clover than NF clover (Table 4).

Precipitation had no effect over the entire growing season on primary growth forage (grasses or clover), except for grass DM yield, which increased 46% ($P = 0.02$) in chambers with high (+20% of average) precipitation compared with average and low precipitation treatments. Although this pattern was similar in the final harvest (Table 4) it was not significant. A precipitation × ozone interaction was also observed for grass DM yield over the growing season. Low (−20% of average) and high precipitation/2× ozone treatment DM yield of grasses increased significantly compared with average precipitation/NF treatments (Table 3). Harvest (month) effects were significant in grasses over the growing season for DM yield and nutritive quality, with both declining in the later harvests.

Re-growth

Grasses exposed to 2× ozone treatments had significantly lower concentrations of NDF throughout the season than NF grasses, higher RFV and increased concentrations of CP than NF exposed grasses through the growing season (Table 5). Re-growth grasses exposed to 2× ozone concentrations had 40% higher DM yield ($P = 0.09$) in the final harvest than NF grasses (Table 6). Grasses also had 5% higher RFV ($P = 0.03$) in the final harvest for 2× ozone compared with NF re-growth grasses. Clover exposed to 2× ozone treatments had higher concentrations of NDF and ADL ($P < 0.03$) over the growing season than NF-exposed clover, resulting in a significant ($P = 0.06$) decrease in RFV in 2× ozone treatments compared with NF treatments over the growing season (Table 5). During the final harvest, 2× clover experienced a 60% decrease ($P = 0.06$) in DM yield compared with NF clover (Table 6). Nutritive quality of the final harvest also significantly decreased in 2× clover, which exhibited an 8% decrease in RFV compared with that of the NF-treated plants.

Precipitation alone did not have significant effects on re-growth forage (grasses or clover) over the growing season or in the final harvest, although high precipitation amounts generally resulted in increased DM yield (Tables 5 and 6). There were significant interactions of DM ($P = 0.001$) and ADL ($P = 0.04$) with precipitation and ozone in grasses (Table 6). The ozone × precipitation interaction resulted in higher DM in grasses exposed to 2× ozone treatments in combination with high and low precipitation amounts compared with NF/average precipitation treatments ($P = 0.001$).

Canopy cover

Total canopy cover did not differ significantly ($P = 0.69$) between ozone treatments. Grasses in general did not differ among ozone treatments but did decrease slightly (not significant) in July and August compared with the earlier months of the study (data not shown). Clover cover also did not differ for ozone treatment ($P = 0.19$) or harvest ($P = 0.38$). Over the growing season, total canopy cover was lowest ($P = 0.001$) in the average precipitation treatments compared with the high and low treatments (93%, 99%, 96%, respectively).

Table 2. Mean daytime (09:00–21:00 h) 12-h ozone concentration (ppb: $nl \cdot l^{-1}$), average ozone peaks, 1-h peaks, AOT 40_12 h [ppm ($\mu l \cdot l^{-1}$)-h] and W126 (ppm-h) concentrations from 1 June–30 September 2009.

	12-h ozone concentration		average ozone peak concentration		Peak 1-h concentration		AOT 40 (ppm-h)		W 126 (ppm-h)	
month	NF	2×	NF	2×	NF	2×	NF	2×	NF	2×
June	35	68	44	91	59	126	742	10.6	0.6	15.8
July	35	61	43	79	73	155	713	8.6	0.7	12.2
August	26	47	34	66	56	113	169	5.0	0.2	6.6
September	26	50	35	73	55	136	188	5.6	0.2	7.9
season	31	56	39	77	73	155	1811	29.8	1.7	42.5

NF = non-filtered ambient, 2× = enriched to twice ambient ozone concentration, AOT40 = accumulated ozone value over a threshold of 40 ppb. W126 = cumulative weighting index (Lefohn *et al.* 1992).

Table 3. Significance (*P*-values) for ANOVA of biomass and nutritive quality for primary growth grasses and clover exposed to differing ozone and precipitation amounts in 2009.

source of variation	df	DM (g)	NDF (%)	ADF (%)	ADL (%)	RFV	CP (%)
primary grasses							
precipitation	2	0.1*	0.75	0.38	0.63	0.62	0.35
O_3	1	0.02**	0.62	0.81	0.89	0.70	0.17
harvest	3	0.001***	0.001***	0.01***	0.11	0.001***	0.001***
O_3 × precipitation	2	0.02**	0.12	0.38	0.61	0.17	0.48
precipitation × harvest	6	0.05**	0.02**	0.02**	0.42	0.02**	0.21
O_3 × harvest	3	0.65	0.16	0.54	0.08*	0.34	0.25
O_3 × precipitation × harvest	6	0.73	0.03**	0.27	0.76	0.05**	0.67
primary clover							
precipitation	1	0.69	0.47	0.79	0.62	0.53	0.46
O_3	1	0.57	0.12	0.12	0.05**	0.13	0.06*
harvest	3	0.004***	0.43	0.01***	0.001***	0.50	0.001***
O_3 × precipitation	1	0.95	0.55	0.23	0.74	0.41	0.91
precipitation × harvest	3	0.39	0.11	0.04**	0.15	0.13	0.86
O_3 × harvest	3	0.83	0.17	0.11	0.30	0.18	0.89
O_3 × precipitation × harvest	3	0.95	0.14	0.12	0.37	0.14	0.99

Significance at $***P < 0.01$, $**P < 0.05$, $*P < 0.10$. DM, dry matter (g); NDF, % neutral detergent fibre; ADF, % acid detergent fibre; ADL, acid detergent lignin; RFV, relative feed value; CP, % crude protein. Due to a lack of clover in average precipitation treatments, chambers with average precipitation were excluded from analysis of clover.

Table 4. Nutritive quality and biomass among ozone and precipitation treatments for final harvest data of primary growth grasses and clover

treatment	DM (g)	NDF (%)	ADF (%)	ADL (%)	RFV	CP (%)
ozone concentration						
grasses						
NF	78.2 (27.2)	69.3 (3.5)	32.9 (2.5)	2.2 (0.4)	85.2 (7.0)	6.8 (1.3)
2×	96.8 (38.8)	69.8 (0.8)	33.4 (1.0)	2.0 (0.3)	83.9 (1.4)	7.1 (1.1)
P-value	0.25	0.73	0.63	0.24	0.63	0.35
clover						
NF	3.5 (3.6)	31.4 (1.6)	18.6 (1.5)	3.4 (0.5)	220.5 (13.6)	19.2 (1.3)
2×	3.3 (3.9)	34.2 (3.5)	20.7 (1.3)	3.8 (0.3)	199.9 (22.8)	20.5 (1.8)
P-value	0.45	0.35	0.15	0.23	0.31	0.10*
precipitation						
grasses						
average	75.8 (24.4)	70.9 (1.2)	33.6 (1.2)	2.0 (0.2)	82.4 (2.5)	5.9 (0.4)
high	120.0 (28.8)	70.4 (1.9)	34.3 (0.4)	2.3 (0.5)	82.2 (2.5)	7.0 (1.2)
low	67.0 (22.8)	67.4 (2.7)	31.5 (2.2)	2.0 (0.1)	89.0 (5.9)	8.0 (0.5)
P-value	0.17	0.21	0.21	0.52	0.20	0.20
clover						
high	1.6 (1.1)	34.2 (3.5)	19.7 (1.9)	3.6 (0.6)	201.8 (24.3)	20.1 (2.2)
low	6.5 (4.2)	31.4 (1.8)	20.0 (1.4)	3.6 (0.1)	218.0 (15.4)	19.9 (0.7)
P-value	0.23	0.43	0.88	0.91	0.50	0.93

Significance at $*P < 0.10$. DM, dry matter (g); NDF, % neutral detergent fibre; ADF, % acid detergent fibre; ADL, acid detergent lignin; RFV, relative feed value; CP, % crude protein. Due to a lack of clover in average precipitation treatments, chambers with average precipitation were excluded from analysis of clover. SD of mean in brackets.

DISCUSSION

To our knowledge, there are no published reports of grassland community responses in the southern USA to increasing ozone concentrations in concert with future precipitation scenarios suggested in several global climate models (MacCracken *et al.* 2001). The ozone concentrations experienced in June–September 2009 were relatively low, approximately 30% lower than those from similar studies in the Auburn area (Powell *et al.* 2003; Szantoi *et al.* 2007, 2009). The average peak values never exceeded 100 ppb (max. 91 ppb), however, the maximum 1-h peak value during the study was 155 ppb in the elevated (2×) ozone treatment. Although ambient rainfall did not fall directly into the chambers, above average rainfall in the area (*ca.* 4 cm more than average) may have affected ozone concentrations due to cloud cover and limited sunlight.

During the experiment, minimal effects of precipitation alone or in combination with ozone were observed. Ambient

Table 5. Significance (*P*-values) for ANOVA of biomass and nutritive quality for re-growth grasses and clover exposed to ozone and different precipitation amounts in 2009.

source of variation	df	DM (g)	NDF (%)	ADF (%)	ADL (%)	RFV	CP (%)
regrowth grasses							
precipitation	2	0.12	0.38	0.40	0.84	0.33	0.25
O_3	1	0.19	0.02**	0.23	0.77	0.03**	0.001***
harvest	3	0.001***	0.001***	0.001***	0.001***	0.001***	0.06*
O_3 × precip	2	0.001***	0.69	0.92	0.04*	0.55	0.83
precipitation × harvest	6	0.77	0.69	0.96	0.14	0.82	0.38
O_3 × harvest	3	0.68	0.85	0.73	0.39	0.58	0.92
O_3 × precipitation × harvest	6	0.84	0.34	0.88	0.34	0.96	0.92
regrowth clover							
precipitation	1	0.65	0.79	0.65	0.62	0.93	0.32
O_3	1	0.64	0.10*	0.14	0.03**	0.06*	0.93
harvest	3	0.002***	0.02**	0.001***	0.001***	0.001***	0.02**
O_3 × precip	1	0.90	0.25	0.53	0.29	0.33	0.31
precipitation × harvest	3	0.82	0.50	0.20	0.39	0.35	0.64
O_3 × harvest	3	0.74	0.18	0.25	0.02**	0.12	0.35
O_3 × precipitation × harvest	3	0.84	0.75	0.75	0.59	0.67	0.74

Significance at ***$P < 0.01$, **$P < 0.05$, *$P < 0.10$. DM, dry matter (g); NDF, % neutral detergent fibre; ADF, % acid detergent fibre; ADL, acid detergent lignin; RFV, relative feed value; CP, % crude protein. Due to a lack of clover in average precipitation treatments, chambers with average precipitation were excluded from analysis of clover.

Table 6. Nutritive quality and biomass among ozone and precipitation treatments, for final harvest data of re-growth grasses and clover.

treatment	DM (g)	NDF (%)	ADF (%)	ADL (%)	RFV	CP (%)
ozone concentration						
grasses						
NF	11.4 (4.0)	68.9 (0.7)	34.8 (0.7)	2.3 (0.3)	83.5 (1.4)	9.39 (0.9)
2×	18.9 (7.9)	67.5 (2.1)	33.8 (1.4)	2.2 (0.3)	88.0 (4.5)	10.1 (0.5)
P-value	0.09*	0.15	0.19	0.44	0.03**	0.13
clover						
NF	2.6 (1.6)	29.0 (1.5)	17.6 (1.6)	3.6 (0.3)	241.3 (14.0)	20.3 (2.2)
2×	1.0 (0.7)	31.2 (2.3)	18.6 (1.3)	3.6 (0.5)	221.9 (21.2)	19.2 (0.5)
P-value	0.06*	0.05**	0.14	0.78	0.03**	0.29
precipitation						
grasses						
average	9.8 (0.3)	68.1 (2.3)	34.7 (1.6)	2.0 (0.2)	84.6 (4.6)	9.9 (0.3)
high	17.1 (10.7)	67.6 (1.7)	33.9 (1.1)	2.5 (0.3)	86.1 (3.3)	10.0 (1.3)
low	18.5 (3.8)	68.9 (0.7)	34.1 (0.7)	2.2 (0.2)	86.6 (4.7)	9.4 (0.5)
P-value	0.33	0.66	0.67	0.29	0.79	0.53
clover						
high	1.5 (1.7)	29.7 (1.4)	17.3 (1.4)	3.7 (0.4)	236.3 (12.8)	20.0 (1.9)
low	2.1 (1.1)	30.5 (1.6)	18.8 (0.4)	3.5 (0.2)	226.8 (12.2)	19.5 (0.6)
P-value	0.62	0.59	0.28	0.56	0.48	0.70

Significance at **$P < 0.05$ and *$P < 0.10$. DM, dry matter (g); NDF, % neutral detergent fibre; ADF, % acid detergent fibre; ADL, acid detergent lignin; RFV, relative feed value; CP, % crude protein. Due to a lack of clover in average precipitation treatments, chambers with average precipitation were excluded from analysis of clover. SD of mean in brackets.

rainfall outside of the chambers was above average for the Auburn area in 2009. Although we attempted to isolate the chambers from ambient rain using rain hoods, precipitation in OTCs may have been affected by water percolation through the soil. Soil moisture measurements were taken starting in June to ensure soil moisture was congruent with rainfall treatments, but measurements were inconsistent and therefore discontinued. Rainfall patterns were calculated on a seasonal basis during this initial effort, which may have influenced the results, because periodicity of rainfall varies during the summer months. These changes in frequency of periods with no rain and higher temperatures during the summer months may be very important, given future climate change scenarios (MacCracken *et al.* 2001; Christensen *et al.* 2007). Changes in the periodicity of rainfall patterns could lead to more frequent droughts and affect productivity of these communities. Main effects of precipitation in the present study should be interpreted with caution, given the initial design of the experiment.

Even with relatively low ozone concentrations, especially in the final 2 months, differences were still observed in some aspects of plant nutritive quality, DM yield and canopy cover of clover. Clover has been reported in several studies as very sensitive to ozone (Blum *et al.* 1982, 1983; Rebbeck *et al.* 1988; Heagle *et al.* 1989b, 1991; Davison & Barnes 1998; Muntifering *et al.* 2006) in terms of nutritional quality and DM yield. We observed significant effects both for primary and re-growth clover in response to elevated ozone concentrations. Primary growth clover, on average, declined in nutritive quality in 2× ozone compared with NF treatments over the harvest months (June–September), with no differences in DM yield. Re-growth clover exposed to 2× ozone treatment declined in nutritive quality over the entire season, but there were no differences in DM yield. However, re-growth clover exposed to 2× ozone treatment did have a significantly decreased DM yield in the final harvest compared with NF treatment. Our results are similar to those from other studies (Blum *et al.* 1982, 1983; Heagle *et al.* 1989b) in which DM yield of both primary and re-growth clover decreased in response to ozone exposure.

Grasses generally did not experience detrimental ozone effects, and even had increases in biomass (DM yield) over the season; although the mechanism for this stimulation is unknown, it has been reported in other systems (Blum *et al.* 1983; Davison & Barnes 1998; Bungener *et al.* 1999; Franzaring *et al.* 2000; Power & Ashmore 2002). Power & Ashmore (2002) examined a grassland system in the UK and found some species had reduced aboveground biomass, others had decreased belowground biomass and one species showed stimulation of aboveground biomass. One possible explanation could involve a shift in partitioning of photosynthate among various plant parts, roots, shoots, foliage, etc. (Rennenberg *et al.* 1996; Pleijel *et al.* 2014). Another explanation is that insensitive grasses appear to fill niches left as the level of the clover falls, similar to the report of Gonzalez-Fernandez *et al.* (2008), who found that ryegrass (*L. perenne*) replaced clover when the two were grown simultaneously under elevated ozone concentrations.

Our results indicate that clover in the grassland community is sensitive to ozone. This response was higher in the re-growth harvests, indicating that timing of harvest may be important in the management of grassland communities under a changing climate. Vegetation composition in communities may alter DM yield and overall community structure such that community-level responses are not fully revealed with simple DM harvests or canopy cover data. These results are similar to those of Blum *et al.* (1983) with a fescue–white clover pasture grown in OTCs in North Carolina. They found that clover was very sensitive to elevated ozone, with fescue replacing clover in the pasture, but after two seasons of exposure the yield reduction in fescue re-growth fell. Nutritive quality of clover (especially re-growth clover) was negatively affected by increasing concentrations of ozone, whereas grasses in general were less sensitive. These data are consistent with other findings (Rebbeck *et al.* 1988; Muntifering *et al.* 2006; Gonzalez-Fernandez *et al.* 2008) that forage species affected by ozone generally contained increased concentrations of cell wall constituents such as NDF, ADF and ADL. Such results, in addition to plant secondary metabolites, will have detrimental effects on nutritional value for herbivores that rely on forage crops such as these for energy (Blum *et al.* 1983; Krupa *et al.* 2004; Gilliland *et al.* 2012).

Cell wall constituents are only partially and variably digestible and have a considerable impact on forage nutritive quality and food value to ruminant herbivores (Van Soest 1994; Powell *et al.* 2003; Krupa *et al.* 2004; Gonzalez-Fernandez *et al.* 2008). According to Van Soest (1994), NDF is inversely related to free-range voluntary forage intake: as forage concentration of the NDF fraction increases, forage intake decreases. Acid detergent fibre is inversely related to forage digestibility; *i.e.* as ADF concentration increases, forage digestibility decreases (Van Soest 1994).

Decreases in nutritive quality due to ozone in clover and other sensitive species such as highbush blackberry (*Rubus argustus*) could have detrimental effects on wildlife that forage on these species (Krupa *et al.* 2004; Ditchkoff *et al.* 2009; Gilliland *et al.* 2012). Since grasses replace much of the area vacated by clover, the overall community nutritive quality could be adversely affected. The nutritive quality of many grasses is much lower than that of clover, and increased fibre content precludes the herbivores from consuming sufficient forage to meet their nutritional needs (Blum *et al.* 1983; Krupa *et al.* 2004; Gilliland *et al.* 2012).

Nutritive quality decline due to increased ozone concentrations with no significant effect on DM yield has been reported in studies with warm season forage crops (Powell *et al.* 2003; Lewis *et al.* 2006). The lack of canopy cover changes due to ozone treatment is important because grass canopy cover did not change significantly but did have higher nutritive quality in 2× ozone treatment than NF treatment. Clover canopy cover also did not differ among ozone treatments, but did a decrease in nutritive quality in 2× ozone treatments compared with NF treatment. This observation can be interpreted as communities appearing unchanged in terms of canopy appearance, but did change in nutritive quality (Muntifering *et al.* 2000; Powell *et al.* 2003; Lewis *et al.* 2006), litter decomposition (Kim *et al.* 1998), N fixation (Montes *et al.* 1983) and/or DM yield (Bungener *et al.* 1999; Franzaring *et al.* 2000; Power & Ashmore 2002). Further research is needed in this area. Also, while there was no visible foliar injury to any plants inside the chambers, DM and nutritive quality were still altered.

The relative insensitivity of grasses to ozone in this study suggests that pooling of grasses into one collective ‘grass’ sample throughout the season was appropriate for testing our hypothesis. The comparison of grasses (insensitive) to clover (sensitive) is important in determining species- or growth-specific responses to different ozone and precipitation treatments (climate change). Community structure and function are important modifiers of the effects of ozone and may alter how individual plants respond to elevated ozone, resulting in changes to species abundance and composition (Barbo *et al.* 1998; Booker *et al.* 2009).

CONCLUSIONS

It is important to determine the effects that altered rainfall amounts combined with different levels of ozone exposure may have on local plant species in the southern Piedmont region of the USA, as well as potential plant/animal interactions (Krupa *et al.* 2004; Gilliland *et al.* 2012) in those communities. Due to design limitations and only one growing season of exposure, further research is needed in this area. However, our results indicate that clover is very sensitive to increasing ozone concentrations regardless of rainfall pattern. In addition, grasses (lower intrinsic nutritive quality) tend to out-compete clover and allocate more resources when grown in competitive environments with such a sensitive species. This may be important for the health and welfare of herbivores that forage on these species.

The southern Piedmont region spans 17 million hectares from central Virginia to east-central Alabama (US Department of Agriculture 1981) and mainly contains managed grasslands and pastures that include C_3 and C_4 grasses and clovers such as the species examined in this study. The differences in ozone sensitivities of species in the southern Piedmont region suggest community-level responses as well as species-specific responses to the combination of altered rainfall amounts and increasing ozone concentrations (Krupa *et al.* 2004; Booker *et al.* 2009). The results described here have management implications in that ruminant herbivores may be forced to alter feeding strategies to account for declines in nutritive quality of forage. Natural declines in forage quality might be further compounded through the detrimental effects of increasing ozone concentrations as rural and forested areas become more urbanised in the future.

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