INSECT COMMUNITY RESPONSES TO SIMULTANEOUS CO_2, TEMPERATURE, AND WATER MANIPULATION WITHIN AN OLD-FIELD ECOSYSTEM

A Thesis

by

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Submitted to the Graduate School

Appalachian State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2007

Major Department: Biology

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ABSTRACT

INSECT COMMUNITY RESPONSES TO SIMULTANEOUS CO_2, TEMPERATURE, AND WATER MANIPULATION WITHIN AN OLD-FIELD ECOSYSTEM (May 2007)

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Climate change researchers have recently recognized a need to shift toward experiments that examine community-level responses to simultaneous exposure of multiple climate change factors (e.g. [CO_2], temperature, and/or water). Previous studies have shown effects of temperature and [CO_2] (especially) individually or in combination on plants and insects, however, little is known about how multiple climate drivers may affect plant-insect community associations. The Old-Field Climate and Atmospheric Manipulation (OCCAM) experiment at Oak Ridge National Laboratory is examining an old-field plant community grown under simultaneous [CO_2], temperature, and water manipulation. My objective was to determine if [CO_2], temperature, water, and their potential interactions would affect both insect community structure, and plant-insect community associations.

I characterized the insect community established on plants at the OCCAM experiment during the 2005 growing season by vacuum sampling and sticky trap collection methods. Insects were identified to morphospecies level to calculate community abundance, richness, evenness, and Shannon-Wiener H'. Insects were

also assigned feeding guilds and diversity calculations were made within guilds to examine potential trophic level effects. I also examined seasonal changes in the insect community and key nutritional components of two dominant plant species.

A mixed model ANOVA found that elevated temperature had the greatest effect on the insect community both among and within season. Examining all morphospecies, I found that temperature alone significantly affected the community by reducing richness, evenness, and H', while not changing abundance. When the most abundant species were considered, my data showed a small number of species increased substantially in abundance at elevated temperature, while others declined compared to ambient temperature. This helped explain effects of temperature on species richness and abundance in the larger data set. Within the herbivore guild, elevated temperature reduced richness and H'. Corresponding reductions of diversity measures at higher tropic levels (i.e. predators and parasitoids) demonstrate trophic-level effects of temperature in my study. This is also supported by my finding that herbivore richness was a significant predictor of parasitoid richness. Elevated [CO_2] and temperature significantly decreased host plant nutritional quality (decreased N and increased C:N), while the potential impact on insect community parameters was seasonally dependent. Non-metric multidimensional scaling and a modified Sørensen similarity index showed that only temperature affected insect community composition. In conclusion, my results demonstrate that an increased temperature can strongly affect insect communities, which could lead to changes in future ecosystem level processes.

ACKNOWLEDGEMENTS

Many people have helped me in countless ways to complete this project, and it gives me great pleasure to express my gratitude for all these gifts. Ray S. Williams advised me with this work, and has also taught me, both inside the classroom and out, how to be a better scientist. I thank Drs. Howard S. Neufeld and Michael Windelspecht for serving on my thesis committee and providing support on my project. They have both always had an open door, as well as, good advice and discussion on any aspect of my project or graduate school in general. Dr. Windelspecht also gave me a unique way to look at my project by allowing me to present my work on a broader scale to his freshman biology class.

Dr. Richard Norby (Oak Ridge National Laboratory) graciously allowed me to collect essential samples from his plant community experiment. Cori Holladay, Bryan Marbert, and Ray S. Williams (Appalachian State University) all provided help in the field with collecting data. E. Cayenne Engel (University of Tennessee at Knoxville) helped coordinate my insect sampling trips with her data collection and maintenance at the OCCAM experiment. Cayenne was also very helpful with providing plant data, along with direction with my initial statistical model. Joanne Ledford (Oak Ridge National Laboratory) graciously analyzed my phytochemical samples. Dr. Larry J. Leamy (University of North Carolina at Charlotte) provided crucial statistical advice as my statistical models became more complex. Dr. Nathan Sanders and Gregory Crutsinger (University of Tennessee at Knoxville) conducted the non-metric multidimensional scaling ordination for me.

I would especially like to acknowledge and thank the many people who did not necessarily help directly with my thesis project, but were nonetheless key in my educational advancement to graduate school. The ecology faculty at the University of North Carolina at Charlotte provided me with the knowledge, experience and confidence to enter graduate school and take on a complex project. Drs. Larry Barden, Larry Leamy, Larry Mellichamp, Ed Menhinick, and Sue Peters are not only excellent teachers and mentors, but each sparked an interest in certain areas of biology that I will have with me always. Dr. Chris Paradise (Davidson College) hired me as a research assistant during the summer before graduate school and provided me with much experience and confidence in conducting primary research in a complex experimental system.

The Cratis D. Williams Graduate School, the Graduate Student Association Senate (GSAS), and the Biology Graduate Student Association (BGSA) all provided funding for my research. My project was part of the OCCAM experiment at Oak Ridge National Laboratory, which is funded by the U.S. Department of Energy Office of Science (Grant No. DE-FG02-02ER63366).

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INTRODUCTION

There is an increasing need to understand how climate change factors may alter terrestrial ecosystem structure and functioning. Increased carbon dioxide concentration ($[CO_2]$) within the atmosphere due to human activities is predicted to result in increased global mean temperatures and regional changes in rainfall patterns. These climate change drivers may independently or interactively affect ecosystem properties such as productivity and species diversity, though much less clear is if the results from studies to date provide the basis to predict whole-ecosystem level responses to multiple climate change factors. Predicting how climate change might affect community structure is crucial for understanding how ecosystem functioning (e.g. carbon (C) sequestration or species assemblages) could change in significant terrestrial systems such as old-field communities, since they represent 10-30% of global C stocks and cover millions of hectares. Therefore, effects of $[CO_2]$ on this system could potentially alter large amounts of global C flux.

$[CO_2]$ Effects on Plants and Insects

Since the Industrial Revolution, $[CO_2]$ has increased to approximately 385 parts per million (ppm) in the atmosphere and is rising by approximately 2 ppm yr^{-1} (National Oceanic & Atmospheric Administration, 2007). There are important implications for plants and ecosystems as $[CO_2]$ increases. Elevated $[CO_2]$ can directly increase photosynthetic rates and Above-ground Net Primary Productivity (ANPP) of both forest (DeLucia *et al.*, 1999; Norby *et al.*, 1999) and grassland (e.g.

Owensby *et al.*, 1999; Reich *et al.*, 2001a) ecosystems, resulting in increased plant biomass (LaMarche *et al.*, 1984). With respect to C flux within ecosystems, changing C allocation patterns in plants may alter tissue quality for insects as the carbon:nitrogen (C:N) ratio increases and foliar N concentration decreases (Coviella & Trumble, 1999). An increased C:N ratio indicates the same quantity of N is now diluted within more leaf tissue, potentially negatively affecting herbivorous insect performance (Hattenschwiller & Schafellner, 2004). Insects confronted with such a decrease in tissue quality often consume more tissue (Lincoln *et al.*, 1993) or increase their nitrogen use efficiency (Williams *et al.*, 1998) to compensate. Even though patterns of increased consumption of elevated $[CO_2]$-grown foliage have been demonstrated, Knepp *et al.* (2005) and Stiling *et al.* (2003) found reduced herbivory in several hardwood species grown under elevated $[CO_2]$. These results could be an example of herbivores actively switching to more nutritious host plants (e.g. 'when in doubt hop about', Bale *et al.*, 2002). In contrast, Veteli *et al.* (2002) did not find a herbivore preference between willow foliage grown under ambient or elevated $[CO_2]$, but did find a significant reduction in growth rate when beetles fed on elevated $[CO_2]$ foliage. Responses to phytochemical changes are not limited to chewing insects. Phloem-feeding aphids show additive negative effects of elevated $[CO_2]$, when allowed to complete several generations, such as laying fewer eggs and reduced larval weight (Brooks & Whittaker, 1998; Whittaker, 2001).

At the community or assemblage level, plant species that demonstrate a response to $[CO_2]$ enrichment when grown alone or in monoculture may not similarly respond, perhaps owing to low densities of the responsive species

(Schappi & Korner, 1996). Morgan *et al.* (2004) report that observed community biomass increase due to [CO_2] was driven mainly by one of 36 species. Species-rich communities may also show a larger magnitude of response to [CO_2] than species-poor communities (Reich *et al.*, 2001b). In this way, intact plant communities can conceal individual species responses because either such responses would not scale-up to community-level changes, or would be mediated by competition between species.

Temperature and Plant-Insect Interactions

As [CO_2] increases, global mean temperature is estimated to increase in concert by 1.4 -5.8°C via the greenhouse effect (IPCC 2001). Previous studies suggest that temperature may be the single most important factor affecting insects in terms of climate change, by altering developmental time, voltinism, population density and size, and distribution (reviewed by Bale *et al.*, 2002). An increase of only a few degrees can accelerate insect development dramatically (Johns *et al.*, 2003; Williams *et al.*, 2003) and cause phenological shifts in development, such as emerging earlier in the year (Masters *et al.*, 1998). Also important to consider is that different species show different degrees of plasticity to temperature increase (Hodkinson & Bird, 2006). Not only are insects physiologically sensitive to temperature, but studies examining the geographic distribution of insects show that they could expand their range latitudinally or to higher altitudes as temperatures rise (Hill *et al.*, 1999; Parmesan *et al.*, 1999; Wilson *et al.*, 2005). Therefore, both

physiological and geographic responses to a warmer climate could potentially occur in many insect species.

Effects of increased temperature on plants are somewhat variable, although some trends are evident. Temperature has profound effects on plant physiological processes, such as growth and development (Rustad & Norby, 2001), but can also indirectly affect plants by increasing soil respiration and net N mineralization (Rustad *et al.*, 2001). Elevated temperature can also affect plant chemical composition by decreasing defensive chemicals (Kuokkanen *et al.*, 2001; Veteli *et al.*, 2002), although the response is somewhat dependent upon the specific compound within the same species (Kuokkanen *et al.*, 2003). In previous climate change experiments, the nutritional quality of foliage, such as N content (Williams *et al.*, 2000; Kuokkanen *et al.*, 2003), water content (Kuokkanen *et al.*, 2001) and C:N (Williams *et al.*, 2000; Johns & Hughes, 2002) has shown no effect of elevated temperature. Aside from phytochemistry, at the community level warming can also accelerate flowering of all species (Price & Waser, 1998) or only one plant species (Engel *et al.*, unpublished data) of a plant community receiving experimental warming treatments. Although warming has a strong direct influence on insects, these previous studies show that temperature can still affect individual and community-level plant characteristics, which may indirectly impact insect communities.

Precipitation

Precipitation patterns can strongly affect ecosystem water availability and energy flow by influencing soil water, temperature, and evaporation, along with soil nutrient availability and mobility (Heisler & Weltzin, 2006). Through these mechanisms, soil water availability can affect a variety of plant physiological processes (Weltzin & McPherson, 2003). Drought can reduce foliar quality (Roth *et al.*, 1997) and soil nutrients, and water availability can also interact to affect constituents of foliar chemistry important to insect herbivores (Lower & Orians, 2002). Within the context of climate change this is important because precipitation patterns are expected to change as global temperatures increase (Hulme *et al.*, 2002). Soil water availability may affect plants indirectly as well. For example, Heisler & Weltzin (2006) suggested that nutrient limitations (due to reduced soil water) may become more influential than the water limitation itself for plants. Changes in plant tissue nutrient concentrations brought about by reduced soil water can also be important for insects. In fact, herbivores were once thought to benefit on water-stressed plants (i.e. the plant stress hypothesis (PSH) White, 1969), because of plant physiological changes, mainly increasing N concentration. However, recent studies have found discrepancies with the PSH. For example, Scheirs & De Bruyn (2005) found no increase in herbivore performance due to water stressed host plants, even though they report an increase in N, while Staley *et al.* (2006) found fewer leaf miners present on drought-stressed plants in their lowland calcareous grassland system, which is also inconsistent with the PSH.

Even though leaf N may increase on drought-stressed plants, herbivorous insects require a mechanism to obtain it. In a meta-analysis examining water stress and plant-insect interactions, Huberty & Denno (2004) found that cell turgor pressure seems to play an important role in determining whether sucking insects can actually benefit from increased nutrient content. Specifically, phloem-feeding insects require positive turgor pressure to enable them to extract fluid (Holtzer *et al.*, 1988; Archer *et al.*, 1995). Also playing an important role is whether plants are intermittently or continuously drought stressed. From the phytochemical standpoint, Huberty & Denno (2004) found that decreases in water content of leaf tissue led to increased toughness and/or secondary metabolite concentration, both of which decrease an insect's ability to access or digest N, respectively. Soil water availability clearly affects plant physiological processes, which could potentially lead to plant community or ecosystem-level changes (Weltzin & McPherson, 2003). To date, studies investigating soil water and other factors have shown much variation across plant taxa and community type (Smith *et al.*, 2000; Shaw *et al.*, 2002). Inconsistent responses and much variation of plant responses to soil water availability both make it difficult to predict how plants or entire communities may respond to future climate (Weltzin *et al.*, 2003).

Studies with Multiple Climate Factors

Studies that incorporate multiple climate change factors likely provide for a more realistic prediction of how plants and insects might be affected because associations between these organisms naturally occur in a multi-factor environment.

Because [CO_2] and temperature are expected to increase simultaneously (IPCC 2001), experiments that incorporate both drivers provide for a better assessment of future effects on ecosystems. In addition to [CO_2] and temperature the importance of precipitation in ecosystem function should be realized (Heisler & Weltzin, 2006).

Studies combining elevated [CO_2] and temperature have shown some general patterns regarding plant-insect associations (Zvereva & Kozlov, 2006). Elevated temperature in conjunction with elevated [CO_2] can offset decreases in herbivore performance (e.g. prolonged development or reduced pupal weight) on plants grown under elevated [CO_2] alone (Veteli *et al.*, 2002), by directly accelerating insect development (Johns & Hughes, 2002). Responses of plant tissue components important to herbivores (such as N and C:N ratio) are somewhat idiosyncratic under elevated [CO_2] and temperature. For example, Williams *et al.* (2003) found no interactive effects of elevated [CO_2] and temperature on foliar N and C:N and Kuokkanen *et al.* (2003) found no combined effects. In a study examining a leaf-mining lepidopteran, Johns & Hughes (2002) found a significant interaction between [CO_2] and temperature on survivorship and adult weight, demonstrating that potentially synergistic effects may occur with multiple climate drivers.

At the plant level, adding an elevated [CO_2] treatment can offset water loss in plants grown under elevated temperature alone because plants can fix C faster, thus enabling their stomates to remain closed for longer periods (Norby *et al.*, 1999; Volk *et al.*, 2000). Similarly, adding soil water manipulation can also affect the response of [CO_2] alone. For example, Huxman & Smith (2001) showed a positive

response of C gain to elevated [CO_2] in plant species only when soil water was readily available. Sorting independent [CO_2] effects from the interactive effects of [CO_2], temperature, or soil water can be difficult. Often, the direct [CO_2] effect disappears when effective soil water is considered (Volk *et al.*, 2000).

Climate Change and Plant-Insect Communities

Studying the factors that shape the connections within plant and insect communities is critical for understanding how those associations may change under future climate. Reich *et al.* (2001a) conducted one of the first experiments examining whole plant assemblages at different diversity levels grown under various levels of [CO_2] and N. This study found that the response to [CO_2] enhancement increased as plant community diversity increased, and that the increases in above-ground biomass at higher diversity levels were due to the most abundant and responsive species. It was also determined that assemblage-level responses were highly dependent upon the number of plant species making up that assemblage. He *et al.* (2002) conducted a complex experiment in which they investigated plant communities related to nutrient and [CO_2] addition across increasing diversity levels. They further differentiated between plant functional groups within their experimental plant communities. Elevated [CO_2] caused a significant increase in plant species evenness due to abundance shifts in plant functional groups. They observed no community-level response to elevated [CO_2] at low or medium diversity levels, but a significant increase in biomass at the high diversity level in agreement with Reich *et al.* (2001a). Studies such as these demonstrate that plant species diversity is

important for determining how responsive a plant community might be to elevated [CO_2], which, in turn, could also affect the productivity of that community (Mulder *et al.*, 2004). Differences in plant species evenness and biomass accumulation could ultimately affect an ecosystem's ability to sequester C or cycle nutrients (Reich *et al.*, 1997; Wilsey & Potvin, 2000). If less responsive species are out-competed within a community, as modeling has predicted (Bolker *et al.*, 1995), elevated [CO_2] could modify the richness and productivity of that community (demonstrated by He *et al.*, 2002).

Plant community diversity can be related to the abundance and richness of heterotrophic species at higher trophic levels (Strong *et al.*, 1984; Hunter & Price, 1992). Insect community richness has been demonstrated to be positively related to plant species richness within grassland ecosystems (Siemann, 1998; Siemann *et al.*, 1998; Knops *et al.*, 1999; Thomas & Marshall, 1999; De Cauwer *et al.*, 2006). Plant richness can also be an important factor affecting insect abundance within grasslands (Koricheva *et al.*, 2000). Mulder *et al.* (1999) reported a positive relationship of plant biomass and herbivory with increasing plant species richness, further suggesting a tight association between plant species richness and insect community characteristics. Other plant community responses, such as biomass, productivity, or functional group richness, have been shown to have weaker correlations with insect community measures (Siemann *et al.*, 1998; Symstad *et al.*, 2000).

Since plant community richness clearly can affect the associated insect community, predicting how future climate may alter plant communities is key to

understanding how it may indirectly affect insect communities. However, few experiments have investigated the effects of climate change on plant community richness in a natural setting (Lloret *et al.*, 2004). He *et al.* (2002) showed that [CO_2] could affect plant community richness. This, along with changes in phytochemistry important to insect herbivores, could be another mechanism by which [CO_2] impacts insects. Increased soil water has also been shown to decrease plant community richness in a southeastern old-field community (Engel *et al.*, unpublished data). Determining generalized conclusions is difficult, as drought increased plant species richness in a Mediterranean-type community (Lloret *et al.*, 2004), perhaps showing responses are dependent upon the community type or species present. Although [CO_2] and soil water have been shown to affect plant community richness, empirical studies of temperature effects on plant richness are still lacking. Moser *et al.* (2005) showed that temperature was an important predictor of plant species richness along an altitudinal gradient, and Francis & Currie (2003) found a correlation between temperature and species diversity on large geographic scales. Aside from altitudinal studies, experimental manipulation of temperature has shown dramatic reductions in species richness due to warming treatments (Klein *et al.*, 2004). These studies suggest that temperature may similarly drive changes in plant community richness, which could in turn affect the associated insect community.

Insect herbivores remove considerable amounts of biomass fixed by plants, and as such can affect plant community composition. Studies have estimated from 2-15% of net primary productivity is removed by herbivores in forest and 4-24% is removed in old-field grassland (Cyr & Pace, 1993; Cebrian, 1999; Hartley & Jones,

2004). Biomass removed by herbivorous insects could at least partially offset biomass gain by plants growing under elevated [CO_2]. At the community level, several studies have shown that insect suppression increased plant community biomass (Mulder *et al.*, 1999; Coupe & Cahill, 2003). It is clear that insects can affect total plant community biomass and biomass allocation (Mulder *et al.*, 1999; Hartley & Jones, 2004), and therefore have the potential to shape plant community composition.

Characteristics of plant communities (e.g., host plant nutritional quality and foliar toughness) undergo seasonal changes that are important for the associated insect community. Plant phenology represents a large source of variation throughout the growing season that can affect phytophagous insects (Feeny, 1970; Schroder, 1986; Kearsley & Whitham, 1989; Bernays & Chapman, 1994). Along with plant phenology, abiotic factors such as temperature and soil water availability, and biotic factors such as herbivory and disease, can affect changes in the nutritional quality of plants throughout the growing season (Bowers & Stamp, 1993; Larcher, 1995). These and other complex interactions may be altered by climate drivers such as elevated [CO_2] which can accelerate (Murray, 1995; Engel *et al.*, unpublished data), delay (Dippery *et al.*, 1995), or not affect (Marc & Gifford, 1984) flowering phenology. By altering plant phenology, climate change factors could also indirectly affect insects that depend on a close synchrony with their host plant (Bale *et al.*, 2002; Dixon, 2003).

Effects of insects on ecosystem processes

When examining how plant or insect communities might change in response to climate change, it is important to focus on factors that are driving trophic levels and their characteristics and interactions. For example, abiotic factors can influence plant and insect communities at the producer level (Price & Hunter, 2005), then move up as nutrients and other materials are transferred to higher trophic levels (Kagata & Ohgushi, 2006). The effects of plant community diversity on insect community composition provide a mechanism to examine trophic level interactions between plants and insects beginning with the plants. Conversely, insect herbivores can affect plant community properties such as succession (Carson & Root, 1999) and plant growth (Moran & Scheidler, 2002) with the result of a higher trophic level exerting effects on a lower trophic level producing community-level regulation (Finke & Denno, 2005).

Understanding trophic level interactions is somewhat dependent upon the ecosystem studied. In old-field insect communities there are three generalized trophic levels: terrestrial plants – insect herbivores – natural enemies (Schmitz, 2003). Several studies have explored this tri-trophic system, although most focused on only two trophic levels (see review by Walker & Jones, 2001). In this system trophic cascades occur if the effects of a particular trophic level are observed in a non-adjacent trophic level, via an intermediate level. An example could be the effects of host plant defensive chemicals reducing fitness in insect natural enemies (Francis *et al.*, 2001). In this example, the trophic cascade involves three linked trophic levels. Both cascades from parasitoids/predators down (Shurin *et al.*, 2002;

Borer *et al.*, 2005) and plants to predators/parasitoids up (Price & Hunter, 2005) have been demonstrated in terrestrial ecosystems.

Because terrestrial systems such as old-field insect communities are represented by several trophic levels, insect species are often grouped into feeding guilds based upon their method of feeding (Root, 1967, 1972). This illustrates a particular species' functional role within the insect community and also allows investigations of specific trophic (guild) levels. For example, herbivore diversity has been shown to correlate more so with predator and parasitoid diversity than with plant diversity (Siemann *et al.*, 1998; Koricheva *et al.*, 2000). Assigning insects to respective guilds also allows for diversity investigations within and among guilds of the insect community (Meyer & Root, 1996; Siemann *et al.*, 1999; Lill & Marquis, 2004; Gruner *et al.*, 2005).

Climate change and natural insect communities

Although much is known about the effects of climate change factors on plants and insects at the individual level, much less is known about how insect communities are affected *in situ*. In forest systems, elevated [CO_2] can reduce foliar herbivory of hardwood species, perhaps due to reduced foliar quality or from increased C-based defensive chemicals (Knepp *et al.*, 2005). Stiling *et al.* (2002, 2003) found reduced herbivory by leaf miners under elevated [CO_2] treatments, likely due to lower herbivore densities inhabiting all plant species within their scrub-oak community. Reductions in herbivory seen in other studies may not translate into changes in the insect community *per se*. For example, Sanders *et al.* (2004) found

that elevated [CO_2] did not affect total arthropod abundance or guild richness within a *Liquidambar*-dominated forest growing under Free-Air Carbon dioxide Enrichment (FACE). Elevated atmospheric [CO_2] itself can also disrupt search cues in an intact aphid-natural enemy system, perhaps by masking important chemicals used as cues (Mondor *et al.*, 2004). For sucking insects at the community level, aphid populations sometimes show no response (Docherty *et al.*, 1997), or increased abundance under elevated [CO_2] (Bezemer *et al.*, 1998).

Very few studies have addressed how multiple climate change factors may affect natural insect communities. Researchers clearly acknowledge the need to address ecosystem processes within the context of multiple factors (Norby & Luo, 2004), and development of experimental designs has improved the ability to regulate multiple factors simultaneously. For example, open-top chambers (OTCs) and FACE provide treatment manipulations in a more natural setting. Because most community-level studies to date have dealt primarily with plant communities and multiple climate change factors, predictions of how the associated insect community may be affected are harder to make. If plant community properties correlate with insect community structure as demonstrated in previous studies (Siemann, 1998; Siemann *et al.*, 1999), indirect effects of climate drivers on plant productivity, diversity, and biomass may also shape insect communities. Therefore, studies that combine multiple factors in intact, natural plant communities can provide great insight into how associated insect communities may be altered as climate changes.

Objectives

This study examined how simultaneous [CO_2], temperature, and soil water treatments affected an old-field insect community, both through the direct effects of temperature on insects, and indirect effects of elevated [CO_2] and reduced water on plant tissue quality.

I addressed four questions in my study:

1. How will [CO_2], temperature, and soil water independently or interactively affect insect morphospecies and guild abundance and diversity in a single growing season?

   Predictions:

   - Elevated temperature will increase insect abundance and diversity across all guilds.
   - Elevated [CO_2] will reduced foliar quality, which will affect the insect community by negatively impacting the herbivorous guild.

2. Will insect community structure show correlations between different trophic levels?

   Prediction:

   - Changes in abundance and diversity in lower insect trophic levels due to treatments will correlate with abundance and diversity of insects within higher trophic levels.

3. Will responses to [CO_2], temperature and water be consistent across sampling dates and with the cumulative insect data?

   Prediction:

   - Changes in the insect community will vary by each sampling date due to large phenological differences in plant and insect communities.

4. Will the insect community show correlations to the plant community via effects of [CO_2], temperature, and water on plant productivity and biomass?

   Prediction:

   - Changes in productivity and biomass of the plant community will positively relate to insect community abundance and structure.

METHODS AND MATERIALS

Experimental Design

My experiment employed modified open-top chambers (Fig. 1) to control [CO_2], temperature, and water conditions as part of the Old-Field Community Climate and Atmospheric Manipulation (OCCAM) experiment at the Oak Ridge National Laboratory (ORNL) Environmental Park. This long-term experiment is examining old-field plant community-level responses to simultaneous applications of [CO_2], temperature, and soil water. The experimental design is a randomized complete block split plot design with four 12.6m^2 open-top chambers each arranged into three blocks (Fig. 2). The chambers are fitted with a precipitation shelter above each chamber to exclude natural precipitation, but remain open to the atmosphere. At the whole-plot level (chamber), [CO_2] (ambient or + 300ppm) and temperature (ambient or + 3°C) are controlled by an air conditioning unit. At the split-plot level, soil water is controlled by applying water in amounts below (dry) and above (wet) natural measured precipitation based on long-term measured weekly precipitation records from nearby Oak Ridge, TN. Each of the three blocks contains one open (unchambered) plot that was constructed in the same manner as chambered plots (Fig. 2). Although the open plots consist of the same plant community as the chambered plots, they do not serve as true controls, because they are exposed to natural precipitation and have no [CO_2] or temperature control.

The plant community within plots consists of seven species common to southeastern temperate old-fields: *Solidago canadensis* L., *Trifolium pratense* L., *Lespedeza cuneata* (Dum. Cours.) G. Don., *Plantago lanceolata* L.,

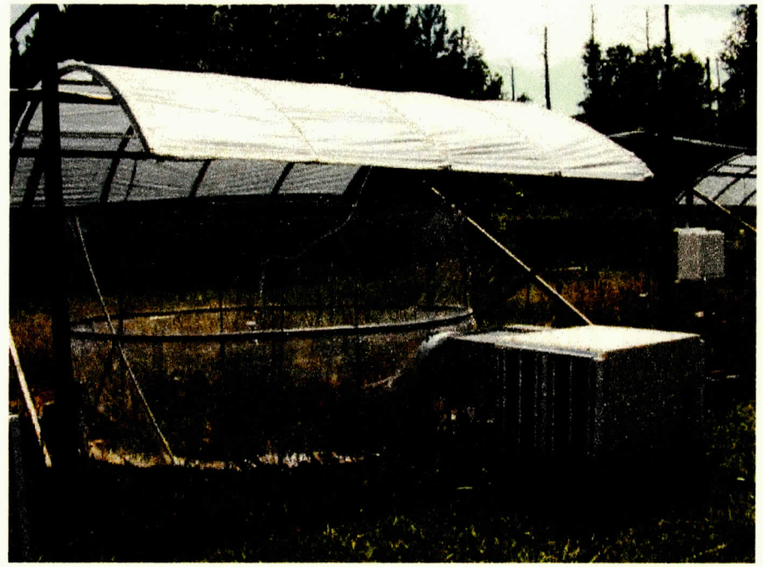

Fig. 1 Modified open-top chamber at the OCCAM site, Oak Ridge National Laboratory, Oak Ridge, TN.

OCCAM Experimental Design

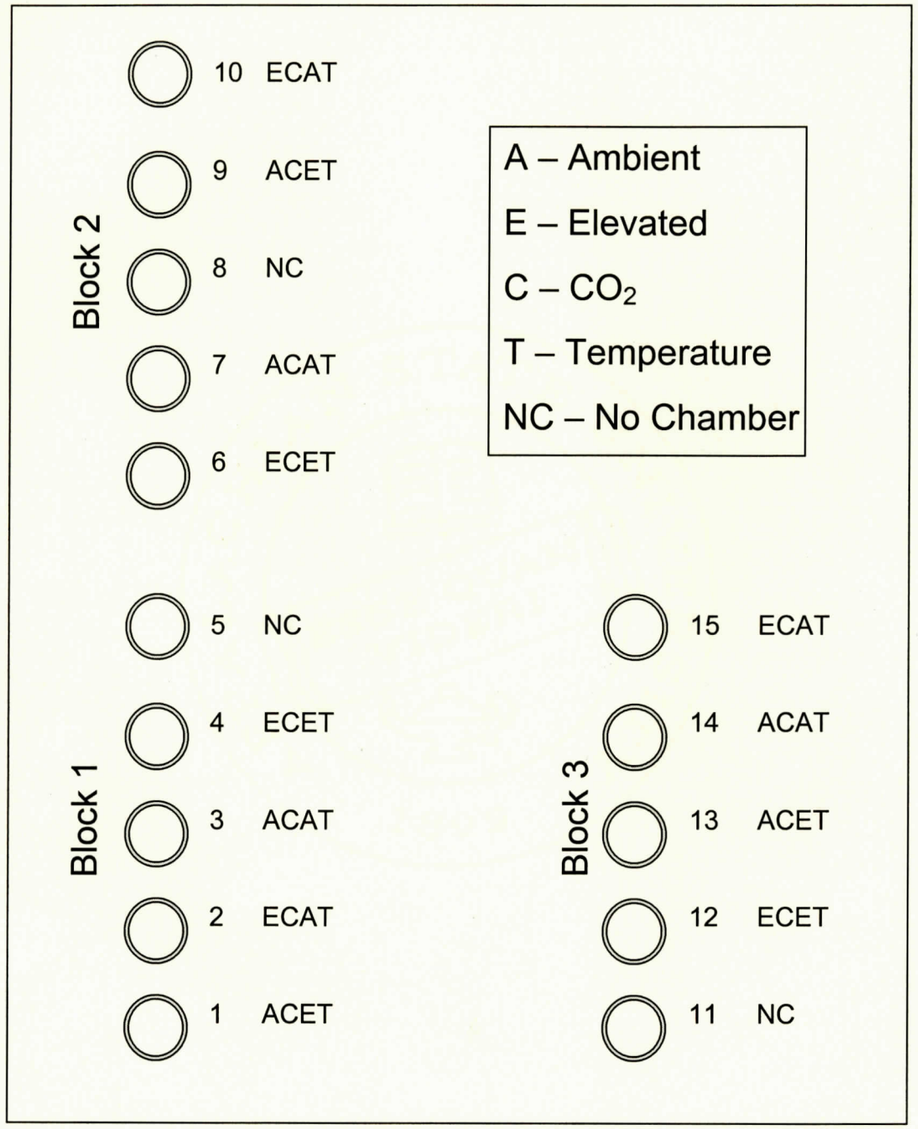

Fig. 2 Diagram showing the experimental layout and treatment assignments of the OCCAM experiment.

Dactylis glomerata L., *Andropogon virginicus* L., *and Festuca pratense* L. syn, *F. elatior* L. Experimental setup and planting were initiated in summer 2002, and experimental treatments began in mid-spring 2003. For detailed information on the setup of the OCCAM experiment see Engel *et al*., unpublished data.

Insect Community Characterization

I sampled the insect community using vacuum and sticky trap sampling methods over the 2005 growing season. Three sticky traps (SureFire™ Consep®, Inc., Bend, OR) were hung above maximum plant height in each subplot for 10 days from 24 June – 3 July and 12 August – 22 August. Traps were collected from the chambers and covered with clear polyethylene in a procedure modified from Hoback *et al*. (1999). The traps were then refrigerated in the laboratory. For the sticky traps alone, I identified insects to Order level due to poor quality of individuals retrieved.

I removed insects using a vacuum sampler three times over the 2005 growing season. On 12 May, insects were collected with a D-VAC® (Rincon-Vitova Insectaries, Inc., Ventura, CA) insect sampler fitted with an extension hose. Within a chamber, each subplot was sampled for 2 minutes to standardize the amount of air pulled through the sampler (following Richardson *et al*., 2002). Care was taken to alternate between which subplots were sampled first within chambers and blocks. The design of this sampler made it difficult to maneuver within chambers without damaging plants, thus a smaller, more compact sampler was used for later samples. For the second (23 July) and third (28 September) samples I employed a

Toro® Rake and Vac™ (Toro, Bloomington, MN) yard vacuum fitted with an insect net covering the intake (similar to Stewart & Wright, 1995). Subplots were again sampled for 2 minutes each, and samples were processed in the same manner at each sampling date. Based on my cumulative dataset from vacuum sampling, both abundance and morphospecies richness were significantly lower ($F_{1,4}$ = 6.09, P = 0.070, and $F_{1,4}$ = 9.74, P = 0.035, respectively) within chambers compared to unchambered plots. Only 13 (3 herbivores, 5 parasitoids, 1 predator, and 4 unknown dipterans) of 163 morphospecies were unique to unchambered plots, which represent 8% of identified morphospecies. Richness and abundance were not substantially different in chambered versus unchambered plots (richness mean = 28 ± 3.33 vs. 39 ± 1.15; abundance mean = 116 ± 15.89 vs. 163 ± 9.86, respectively). Although open-top chambers have been suggested to restrict movement of herbivorous insects (Richardson *et al.*, 2000), the presence of 150 distinct morphospecies within chambers suggests minimal chamber effects on insect colonization and movement.

Upon collection, the contents of each vacuum sample were emptied into a gallon-size freezer bag and chilled onsite. Upon returning to the laboratory, samples were frozen at -80ºC for future identification. Insects were sorted from associated plant debris using an Olympus® SZ40 stereoscope (Olympus, Tokyo, Japan) at 10X and stored in 95% ethanol. Insect specimens were initially identified to Family level following Borror & White 1970; Slater & Baranowski 1978; White 1983; Borror *et al.* 1989. Following Family identification, insects were further identified to morphospecies level using external characteristics and morphology alone (following

Sanders *et al.*, 2004). Morphospecies-level identification is a technique often used for very speciose groups (e.g. insects) as a surrogate to formal, Latin binomial identification (Oliver & Beattie, 1993, 1996). Specifically, individuals within the same Family were arbitrarily named as each morphologically-dissimilar individual was encountered (Appendix). Identified and named morphospecies were immediately photographed and added to an electronic database. The photographs were also printed, with the name attached, to assist in comparisons of subsequent individuals. Identified morphospecies were assigned to a feeding guild (herbivore, detritivore, predator or parasitoid) to examine functional community structure (Bassett & Arthington, 1992; Hodkinson *et al.*, 2004; Appendix).

I characterized insect community composition by quantifying abundance and calculating diversity indices: richness, evenness, and Shannon-Wiener H'. Specifically, morphospecies richness is the number of species collected (Whittaker, 1999). Evenness was calculated as Evenness=H'/ $\log_e S$, where S is the number of morphospecies within each subplot (Rieske & Buss, 2001). Shannon-Wiener H' was calculated as $H'=\Sigma p_i \log_e p_i$, where p_i is the number of individuals within each morphospecies i, divided by the total number of individuals. In order to account for the effects of abundance on species richness, I used rarefaction to examine the responses of rarefied insect richness to experimental treatments (EcoSim, Gotelli & Entsminger, 2001). For abundance, richness, evenness, and H' the subplot served as the experimental unit. Each variable was quantified at the cumulative morphospecies levels and within feeding guilds to investigate community-level changes in insect community structure (Sanders *et al.*, 2004; Andrew & Hughes,

2005). I also analyzed abundance, richness, evenness, and Shannon-Wiener H' within and among sampling dates to investigate variation in the insect community both between and within season.

Phytochemistry Samples

In order to assess treatment effects on phytochemical constituents important to insects, I sampled plant tissue two times over the 2005 growing season for two dominant plant species. On 12 May I marked two individuals of *S. canadensis,* and *L. cuneata,* within subplots in order to follow individuals and quantify phytochemical constituents early and late in the season. On this date I collected 2 leaves for *S. canadensis*, 12 leaves for *L. cuneata* to analyze for total N, C:N, leaf water, and carbon-based phenolics. Another set of N, C:N, and phenolic leaf samples was collected on 28 September in the same manner. On both dates samples for N, C:N, and water analyses were immediately weighed and leaf area measured using a LiCor LI-3100 leaf-area meter (Li-Cor, Inc., Lincoln, NE). Leaves were transferred to envelopes, dried in a drying oven at 40ºC to constant weight, then ground in an amalgamator (Darby Dental Co., East Lansing, MI). This tissue was analyzed for total C and N using an elemental analyzer (CE Elantech, Inc., Lakewood, NJ). I calculated water content as the difference in fresh and dry weight of leaves, and specific leaf area and specific leaf nitrogen as weight or N content per unit area, respectively. Previously frozen leaves for C-based phenolics analysis in *S. canadensis* and *L. cuneata* only were used due to low and inconsistent tissue availability in the other less dominant species. Calculated total phenolics are

expressed as percent tannic acid equivalents (%TAE) and not absolute quantity following the protocol of Singleton & Rossi (1965).

Statistical Analyses

I used the Restricted Maximum Likelihood (REML) algorithm of Proc Mixed (SAS version 8.02, SAS Institute Inc., Cary, NC) to analyze main and interactive effects of treatments on insect abundance, diversity indices (all morphospecies and within guilds) and plant phytochemistry. Carbon dioxide, temperature, and water were set as fixed effects factors and block as a random effect in the model (Littell *et al.*, 1996). Data were natural-log transformed to improve homogeneity of variances as needed. Denominator degrees of freedom were estimated using the Kenward-Roger method (Kenward & Roger, 1997; Spilke *et al.*, 2004, 2005). Due to the low replication inherent in this experimental design, P – values ≤ 0.10 were set *a priori* as significant (Filion *et al.*, 2000) to minimize the probability of making a type II error (Morgan *et al.*, 2005), thus increasing statistical power. Insect community abundance and diversity measures were analyzed in two ways: cumulatively (i.e. by combining all sampling dates) and by including date as a repeated measure, then performing all analyses within each sampling date.

In the cumulative dataset I used a modified Sørensen index to examine similarity of species richness within communities defined by main treatments (e.g. comparing communities under ambient versus elevated temperature; Chao *et al.*, 2005). Regression (Proc Reg, SAS) was used to examine relationships between the plant and insect community where insect abundance and richness are dependent

variables and Annual Net Primary Productivity (ANPP) and total plant community biomass are independent variables. In cases where treatment effects on morphospecies richness were dependent on abundance (based on rarefaction results), expected richness was used in place of actual richness as a dependent variable. For trophic level relationships expected predator and parasitoid richness served as dependent variables and expected herbivore richness as an independent variable. Non-metric multidimensional scaling (NMS) (Primer, Clarke & Grant, 2001) was used to assess the dimensionality of community data by examining the overall dataset compared to main treatments (McCune & Mefford, 1999).

Because temperature alone affected several measures of the insect community, a dataset which accounted for approximately 90% of the total morphospecies abundance at ambient temperature was created (hereafter dominant morphospecies). Morphospecies were ranked by order of abundance based on the ambient temperature treatment and presented with the corresponding morphospecies abundance at elevated temperature. This provided an abundance measure individually for each of the dominant morphospecies both at ambient and elevated temperature. The percent change from ambient temperature for each of the dominant morphospecies was calculated. Finally, richness, evenness, and Shannon-Wiener H' were calculated and compared between ambient and elevated temperature treatments in the dominant species data set using Proc Mixed (SAS) to further elucidate the effects of temperature on the insect community.

RESULTS

Insect Community

Sticky Traps

Sticky traps collected a total of 35,622 insects (from 10 Orders), over the two sampling periods (Table 1), with the thrips, Order Thysanoptera, being numerically dominant.

Table 1 Total insect abundance within respective Orders collected with sticky traps

Order	Count	% of Total
Thysanoptera	30,693	86.2
Hymenoptera	2,155	6.05
Diptera	1,512	4.24
Coleoptera	619	1.73
Homoptera	347	0.96
Lepidoptera	227	0.63
Hemiptera	61	0.17
Psocoptera	5	0.01
Orthoptera	2	0.01
Odonata	1	<0.01
Total	**35,622**	

In three of 10 Orders collected, there was a significant effect on abundance for [CO_2], temperature, or water. Elevated temperature increased the abundance of Thysanoptera by 122% ($F_{1,12}$ = 8.98, P = 0.095; Fig. 3a), while elevated [CO_2] decreased Diptera abundance ($F_{1,4}$ = 17.84, P = 0.019; Fig. 3c). Homoptera ($F_{1,8}$ = 7.53, P = 0.025; Fig. 3b), and Diptera ($F_{1,12}$ = 7.15, P = 0.020; Fig. 3c) abundance were increased by 91% and 42%, respectively in subplots with sufficient soil water.

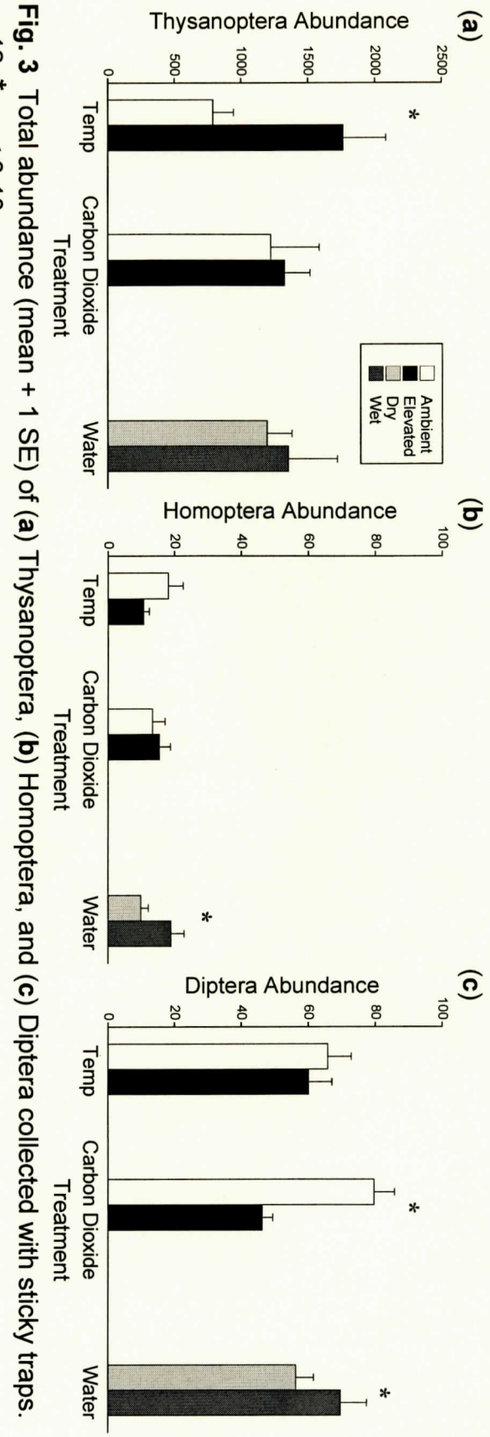

Fig. 3 Total abundance (mean + 1 SE) of **(a)** Thysanoptera, **(b)** Homoptera, and **(c)** Diptera collected with sticky traps. n=12; * = $p \leq 0.10$.

Cumulative Morphospecies

Over the three sampling dates, vacuum sampling collected a total of 4,094 insects, representing 163 distinct morphospecies in four distinct guilds (Table 2).

Table 2 Total insect abundance in guilds collected with vacuum sampling

Guild	Count	% of Total
Herbivore	2,092	51.1
Predator	986	24.1
Parasitoid	628	15.3
Detritivore	373	9.13
Unknown	15	0.37
Total	**4,094**	

The effects of $[CO_2]$, temperature, water, and their interactions on insect community parameters for cumulative morphospecies and feeding guilds are presented in Table 3 and Figs. 4-9. Cumulative morphospecies abundance was unaffected by $[CO_2]$, temperature, water, or their interactions (Table 3; Fig. 4). Richness and evenness were unrelated to $[CO_2]$, or water treatments (Table 3), but significantly declined by 15% and 13%, respectively at elevated temperature compared to the ambient temperature plots (Table 2; Fig. 5a-b). Shannon-Wiener H' was significantly lower at elevated temperature but was unaffected by either $[CO_2]$ or water (Table 3; Fig. 5c).

Table 3 *P* – values and *df*[1] (Proc Mixed) for main and interactive effects on diversity measures for cumulative morphospecies and guilds. *P* – values in **bold** ≤ 0.10; n=12

	CO_2	Temp	Water	CO_2 x Temp	CO_2 x Water	Temp x Water	CO_2 x Temp x Water
Cumulative Morphospecies							
Abundance	0.330	0.496	0.782	0.880	0.705	0.662	0.601
Richness	0.357	**0.045**	0.963	0.679	0.889	0.301	0.230
Evenness	0.324	**0.044**	0.357	0.920	0.556	0.412	0.963
H'	0.595	**0.030**	0.346	0.985	0.511	0.609	0.685
Guilds							
Herbivore							
Abundance	0.251	0.695	0.647	0.670	0.604	0.892	0.676
Richness	0.663	**0.012**	0.999	0.904	0.345	0.551	0.407
Evenness	0.263	**0.069**	0.599	0.995	0.414	0.998	0.836
H'	0.313	**0.003**	0.439	0.976	0.129	0.790	0.576
Predator							
Abundance	0.916	**0.007**	0.867	0.383	0.966	**0.050**	**0.054**
Richness	0.134	0.670	0.999	**0.049**	**0.017**	0.962	0.962
Evenness	0.882	0.591	0.758	0.238	0.211	**0.049**	0.474
H'	0.309	0.687	0.389	**0.032**	**<0.001**	**0.013**	0.435
Parasitoid							
Abundance	0.629	0.541	**0.057**	0.261	0.608	0.373	0.731
Richness	0.198	**0.026**	0.904	0.904	0.407	0.199	0.407
Evenness	0.884	0.166	0.206	0.783	0.931	0.847	0.692
H'	0.323	**0.005**	0.516	0.210	0.649	0.420	0.450
Detritivore							
Abundance	0.211	**0.039**	0.618	0.269	0.853	0.582	0.812
Richness	0.827	**0.097**	0.827	**0.045**	0.515	0.663	0.663
Evenness	0.719	0.299	0.647	0.697	0.712	0.159	**0.011**
H'	0.832	**0.096**	0.947	0.240	0.439	0.801	0.791

[1] *df* =1,4 for *cumulative morphospecies*: abundance, H' CO_2, temperature, CO_2 x temperature; *detritivore*: abundance, H' temperature. *df* =1,8 for *cumulative morphospecies*: abundance, H' water, CO_2 x water, temperature x water, CO_2 x temperature x water; evenness all terms; *detritivore*: evenness all terms. *df* =1,2 for *cumulative morphospecies*: richness CO_2, temperature; *detritivore*: richness temperature. *df* =1,10 for *cumulative morphospecies*: richness CO_2 x temperature, water, CO_2 x water, temperature x water, CO_2 x temperature x water. *df* =1,12 for *detritivore*: abundance, richness, H' CO_2, CO_2 x temperature, water, CO_2 x water, temperature x water, CO_2 x temperature x water. *df* = 1,16 for *herbivore*, *predator*, *parasitoid*: abundance, richness, evenness, H' all terms.

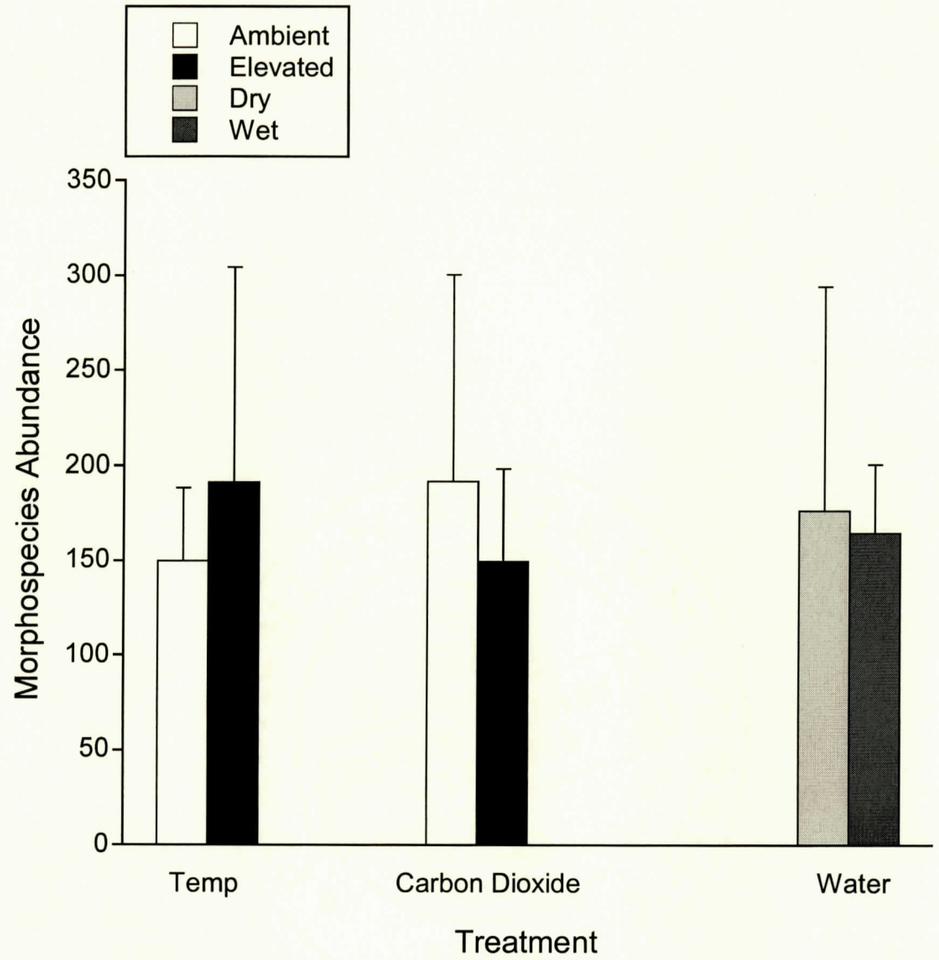

Fig. 4 Cumulative morphospecies abundance (mean + 1 SE) by treatment. n=12.

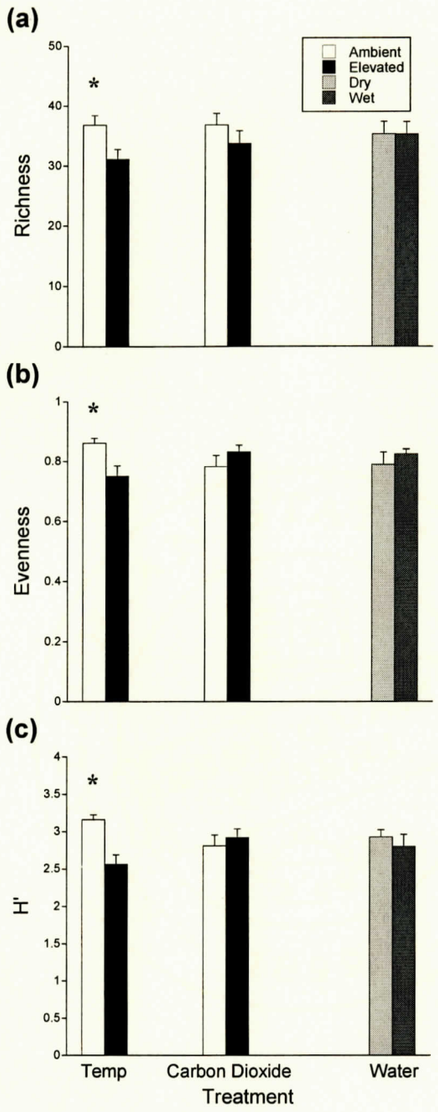

Fig. 5 Cumulative morphospecies (mean + 1 SE) (**a**) richness, (**b**) evenness, and (**c**) Shannon-Wiener H'. n=12; *****= $p \leq 0.10$. *P* – values generated by Proc Mixed (SAS).

Guild Structure

Four major guilds were represented by insects removed by vacuum samples (Table 2). The addition of [CO_2] had no effect on the abundance of any guild, while the abundance within certain guilds was affected by temperature and water (Table 3; Fig. 6a-c). Herbivore abundance was higher at the elevated temperature, while predator abundance significantly increased by 50% and detritivore abundance significantly decreased by 47% at higher temperature (Fig. 6a). Parasitoid abundance decreased by 26% in the dry treatment (Fig. 6c), and there was a significant temp x water and CO_2 x temp x water interaction for predator abundance (Table 3).

Elevated temperature significantly reduced cumulative morphospecies richness by 23% in herbivores, 36% in detritivores, and 22% in parasitoids compared to the ambient temperature treatment (Fig. 7a). Morphospecies richness was unaffected by [CO_2] or water in all guilds (Table 3; Fig. 7b-c). Predator richness, however, was not significantly reduced (as in the other 3 guilds) which could be because the magnitude of the temperature effect was different for high and low [CO_2], which led to a significant CO_2 x temp interaction. Detritivores did show a significant CO_2 x temp interaction for richness because the magnitude of the reduction depended on [CO_2] level (Table 3; data not shown).

Evenness was unaffected in any guild by [CO_2] or water, while in the herbivores there was a 19% reduction in evenness at elevated temperature (Table 3; Fig. 8a-c). Within the predator guild there was a significant temp x water

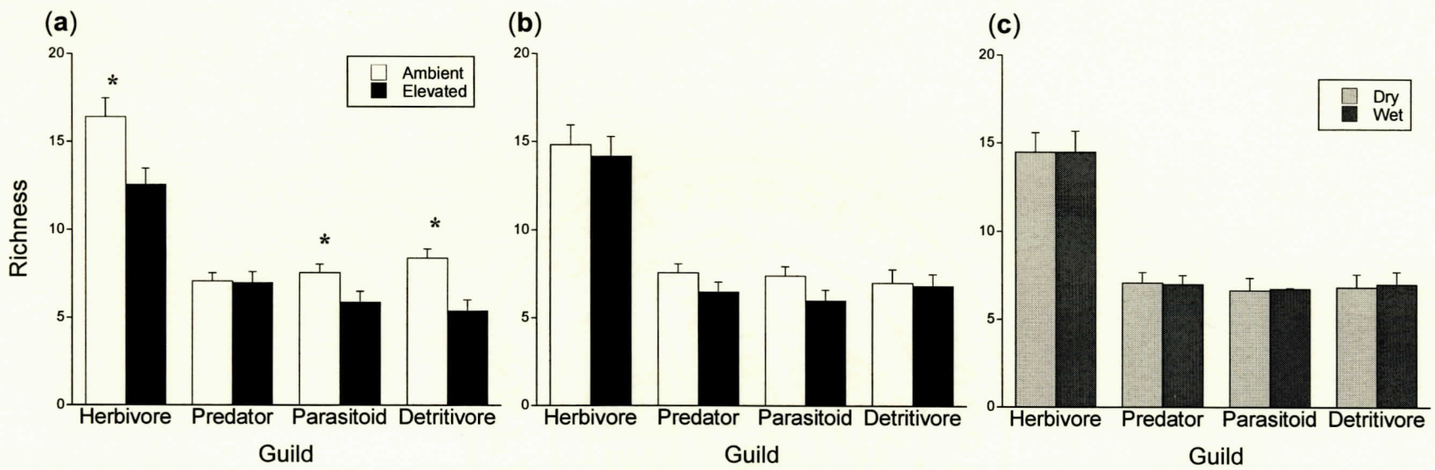

Fig. 7 Cumulative morphospecies richness (mean + 1 SE) within guilds for (**a**) temperature, (**b**) carbon dioxide, and (**c**) water. n=12; *= $p \leq 0.10$.

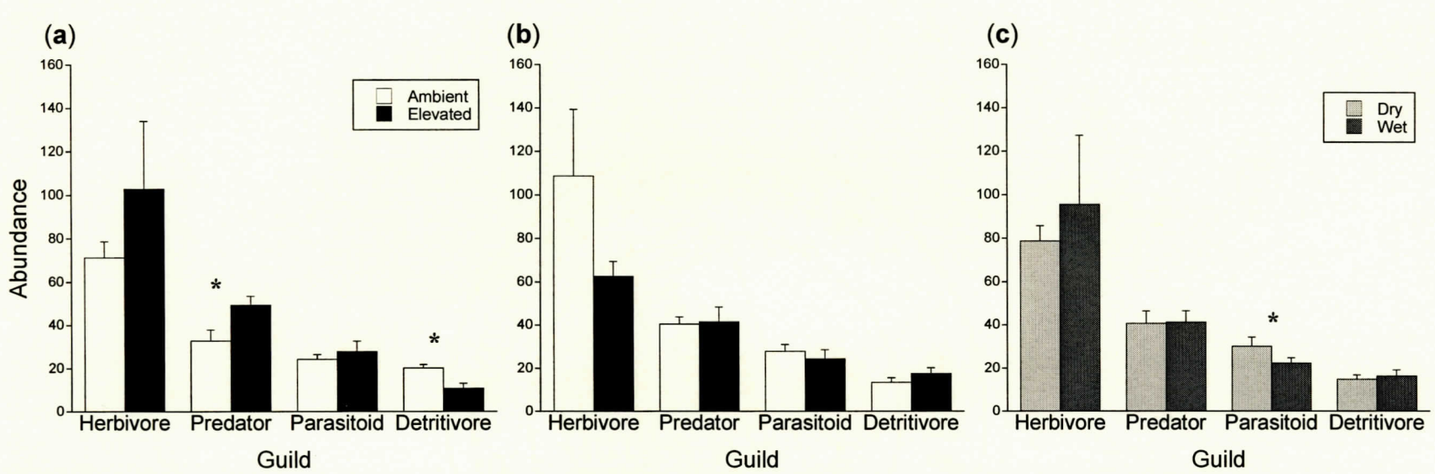

Fig. 6 Cumulative morphospecies abundance (mean + 1 SE) within guilds for (**a**) temperature, (**b**) carbon dioxide, and (**c**) water. n=12; *= $p \leq 0.10$.

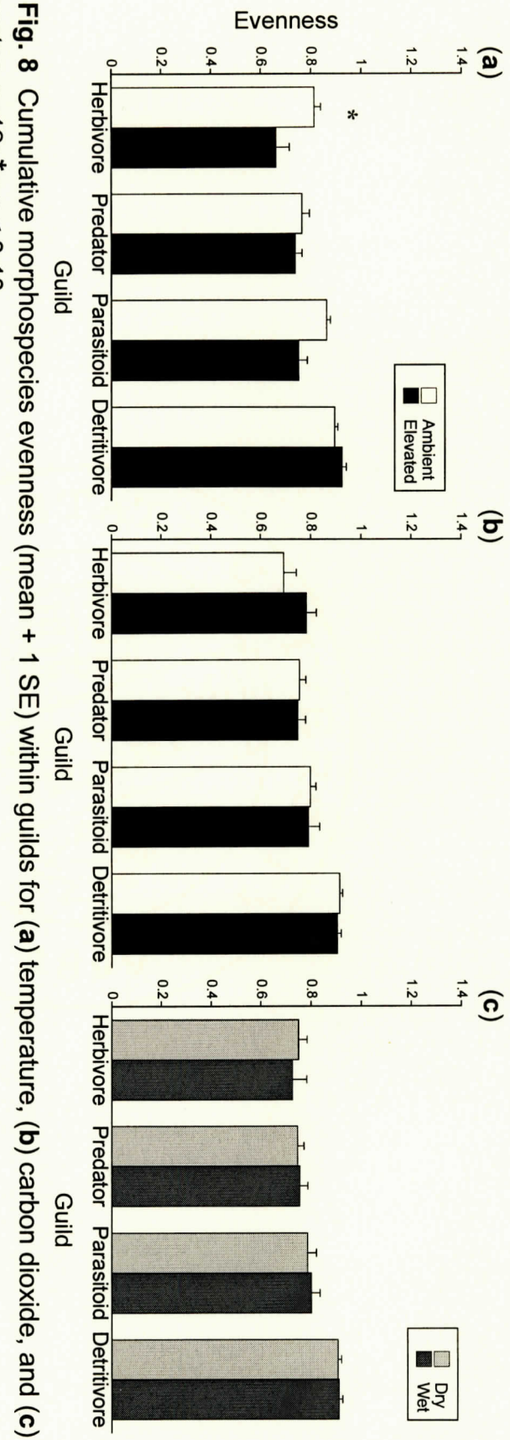

Fig. 8 Cumulative morphospecies evenness (mean + 1 SE) within guilds for (**a**) temperature, (**b**) carbon dioxide, and (**c**) water. n=12; *= $p \leq 0.10$.

interaction on evenness owing to a slightly higher value at the ambient temperature under wet treatments (Table 3; Fig. 8a-c).

Elevated temperature decreased Shannon-Wiener H' of herbivores, detritivores and parasitoids (Fig. 9a), while neither [CO_2] nor water were related to this measure (Table 3; Fig. 9b-c). Predators had significant CO_2 x temp, CO_2 x water, and temp x water interactions for Shannon-Wiener H' (Table 3).

The relative abundance-based Sørensen index for the cumulative morphospecies showed a 91.4% similarity between insect communities at elevated versus ambient temperature (Table 4). Overall, the number of species shared between temperature treatments was less than that of either [CO_2] or water treatments. Both the similarity for [CO_2] (94.1%) and water (96.9%) were greater than that of temperature (Table 4), suggesting more separation in community composition due to temperature alone in this experiment.

Table 4 Sørensen Index[1] for cumulative morphospecies abundance

	Number Species	Total Abundance	Number Shared Species	Index Value
Treatment Comparison				
AT vs. ET	130 vs. 104	1669 vs. 2067	83	0.914
AC vs. EC	115 vs. 127	2129 vs. 1716	90	0.941
Wet vs. Dry	123 vs. 118	1915 vs. 2053	89	0.969
Dominant Morphospecies				
AT vs. ET	38 vs. 38	1544 vs. 2084	36	0.988

AT – Ambient Temperature, ET – Elevated Temperature
AC – Ambient [CO_2], EC – Elevated [CO_2]
[1] Sørensen index modified by Chao *et al.* (2005).

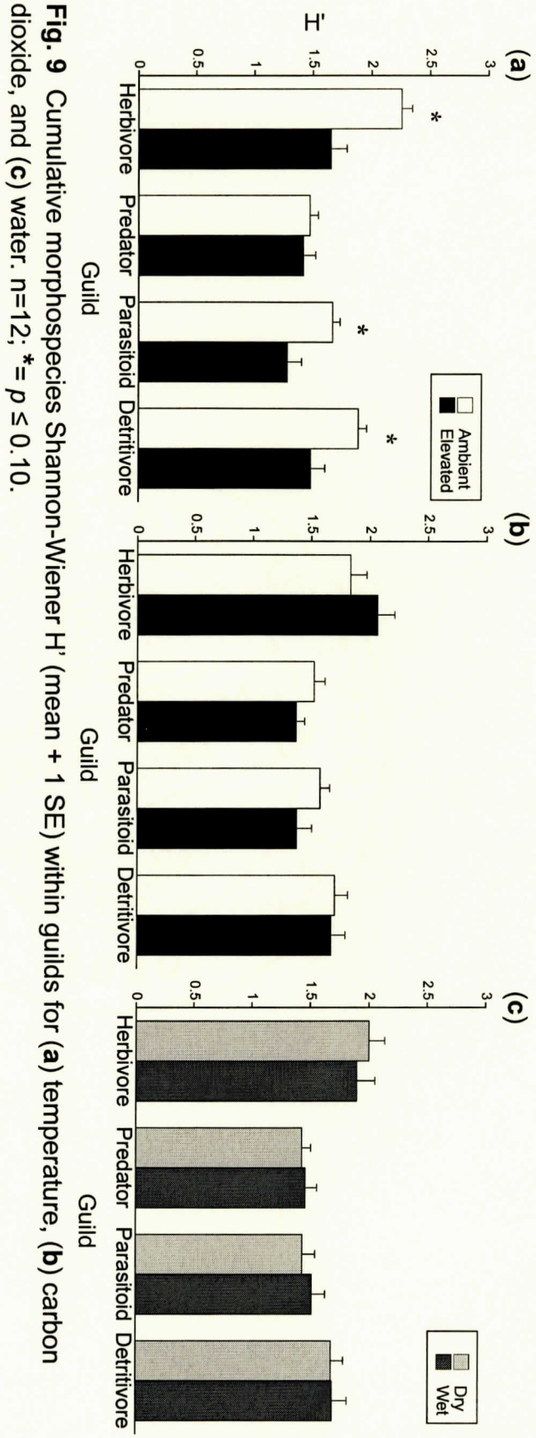

Fig. 9 Cumulative morphospecies Shannon-Wiener H' (mean + 1 SE) within guilds for (**a**) temperature, (**b**) carbon dioxide, and (**c**) water. n=12; *= $p \leq 0.10$.

In order to account of the effects of abundance on morphospecies richness, I used rarefaction (EcoSim, Gotelli & Entsminger, 2001) to generate expected richness values for each subplot. When calculated expected values were compared between treatments (Proc Mixed, SAS), there were no significant relationships between temperature ($P = 0.110$), [CO_2] ($P = 0.558$), or water ($P = 0.675$) and morphospecies richness, demonstrating that the significant effect of temperature on species richness in my experiment is due to individuals.

Non-metric multidimensional scaling showed a strong effect of temperature on cumulative insect community composition (Global $R = 0.311$, $P = 0.001$; Fig. 10). Neither [CO_2] nor water showed any effect on cumulative insect community composition (Global $R = 0.012$, $P = 0.387$ and Global $R = -0.032$, $P = 0.687$, respectively; Figs. 11, 12).

Dominant morphospecies

The rank order of the 38 most abundant morphospecies at the ambient and elevated temperature is found in Fig. 13. Examining the magnitude and direction of dominant morphospecies percent change from ambient temperature reveals that 10 of the 38 morphospecies increased at an elevated temperature relative to the ambient, with 6 by more than 100% (Fig. 14). Interestingly, analysis of the dominant morphospecies based on temperature showed similar effects as that of the full dataset. There was no effect of temperature on abundance ($F_{1,4} = 0.89$, $P = 0.399$). Richness, however, decreased by 18% at elevated temperature compared to ambient temperature ($F_{1,14} = 14.90$, $P = 0.002$). Both evenness ($F_{1,6} = 9.58$, $P =$

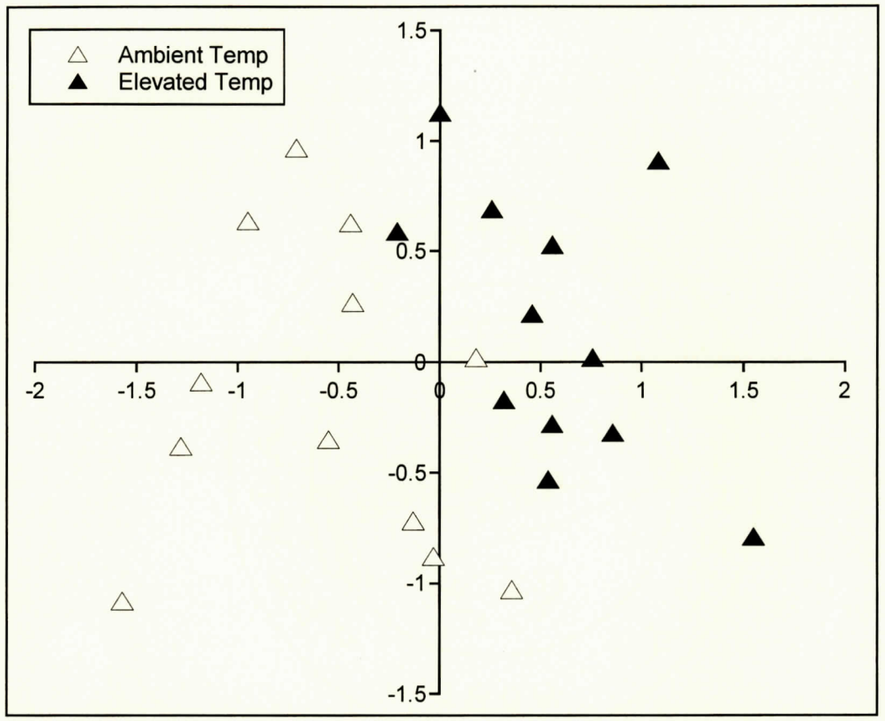

Fig. 10 Non-metric multidimensional scaling ordination of the cumulative insect community composition at ambient temperature (open triangles) and elevated temperature (solid triangles). n=12.

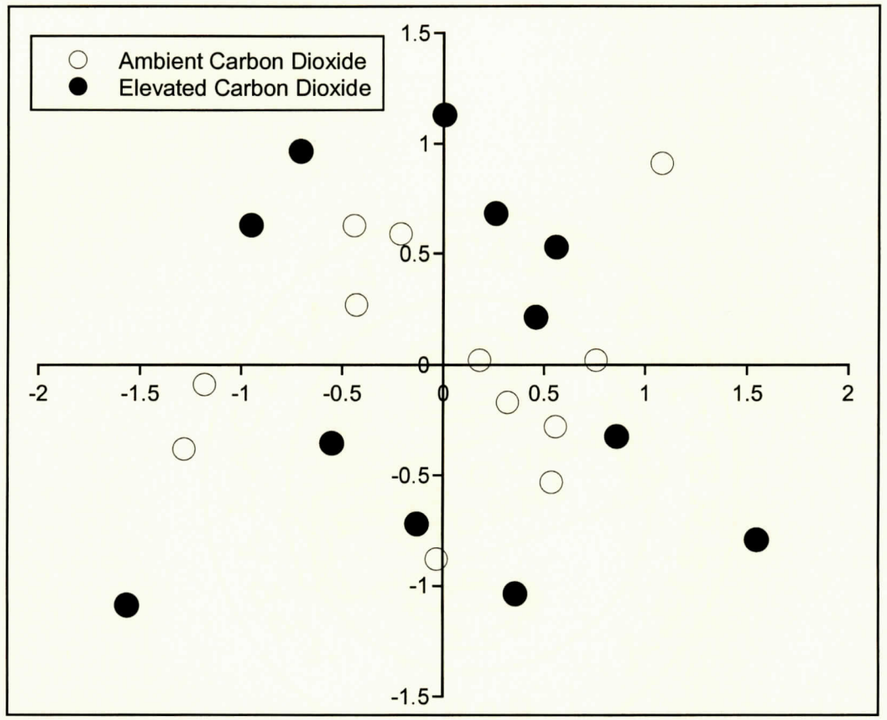

Fig. 11 Non-metric multidimensional scaling ordination of the cumulative insect community composition at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). n=12.

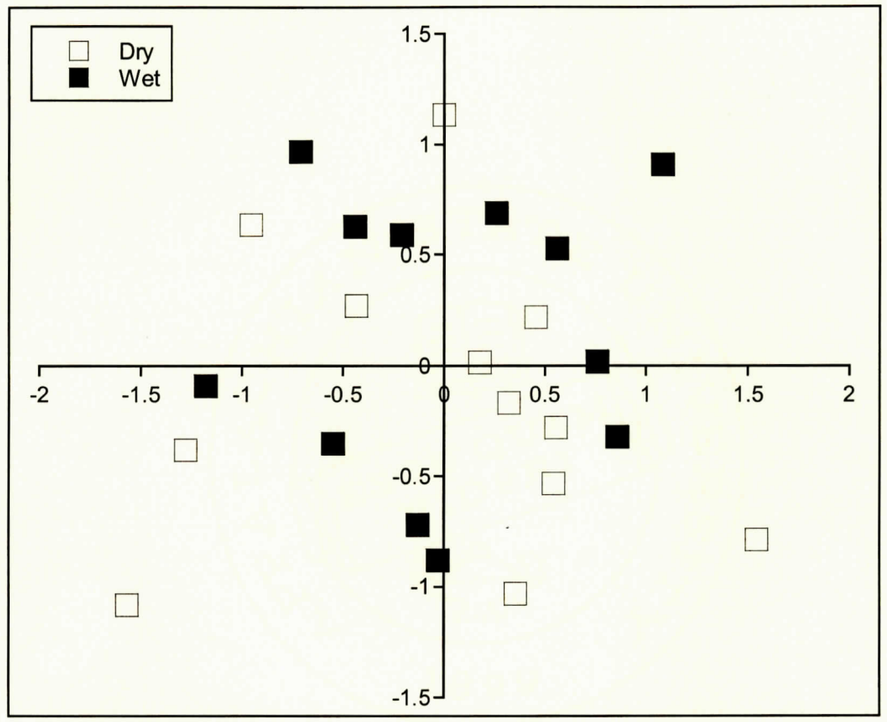

Fig. 12 Non-metric multidimensional scaling ordination of the cumulative insect community composition for dry (open squares) and wet (solid squares) treatments. n=12.

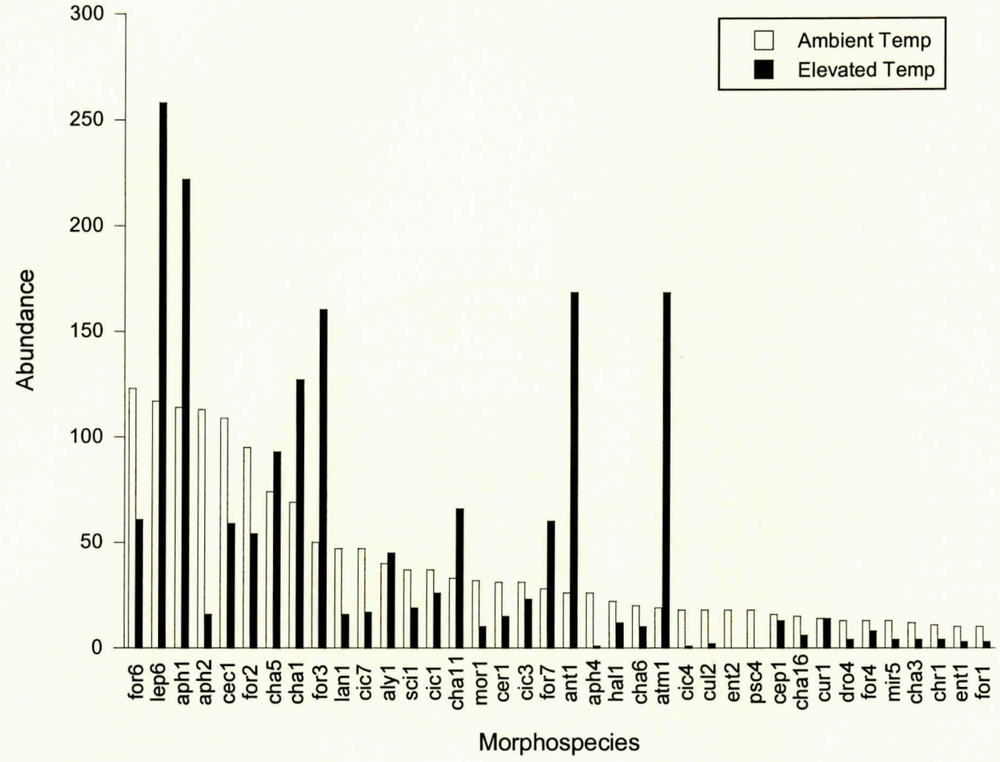

Fig. 13 Rank order of individual morphospecies at the ambient temperature (from most to least abundant) for ambient and elevated temperature. n=12; *= $p \leq 0.10$.

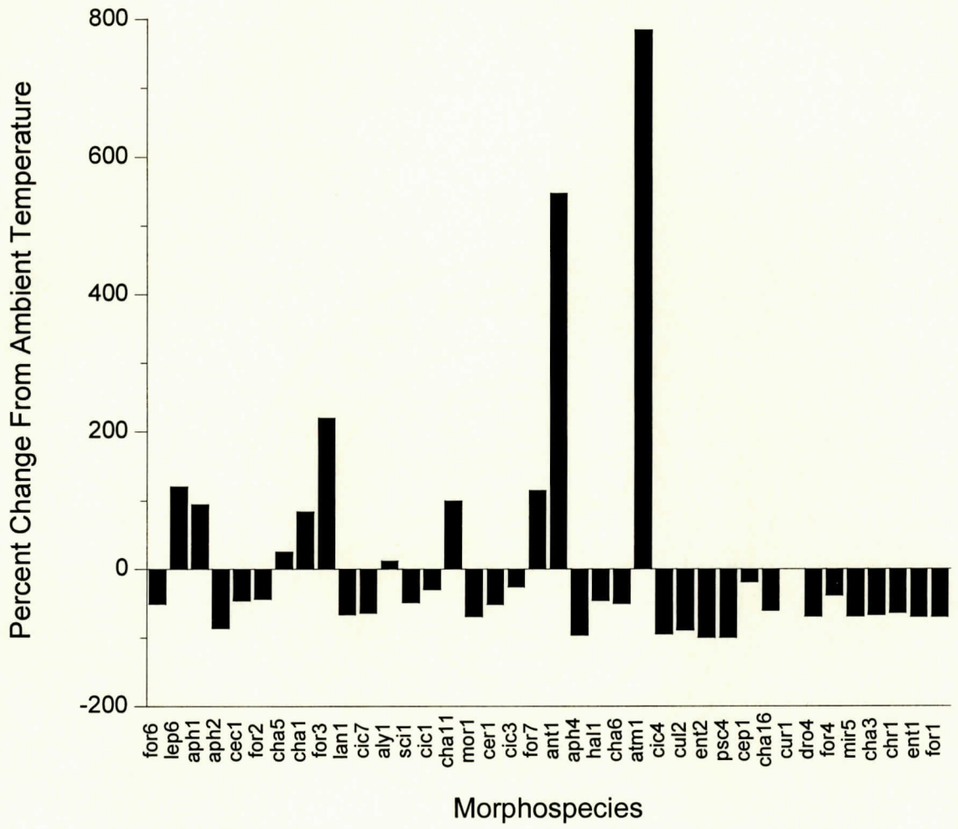

Fig. 14 Percent change in total abundance at elevated temperature from ambient temperature for the dominant morphospecies.

0.021) and Shannon-Wiener H' also decreased ($F_{1,14}$ = 28.30, P = 0.001) under elevated temperature. Of the 38 dominant morphospecies, four significantly increased at elevated temperature (Group/Guild; Microlepidoptera/herbivore, Formicidae/predator, Anthocoridae/predator, and Chalcidoidea/parasitoid; Fig. 15). Five morphospecies were significantly lower at elevated compared to ambient temperature (Group/Guild; Aphididae/herbivore, Ceratoponidae/predator, Culicidae/detritivore, Lathrididae/detritivore, and Mordellidae/herbivore; Fig. 15). The abundance-based Sørensen index showed a 98.8% similarity between ambient and elevated temperature for the dominant morphospecies dataset (Table 4).

Seasonality

The number of insects collected increased throughout the growing season, with the most collected in September, irrespective of treatment (Fig. 16). With all sample dates considered there was a significant effect of date, [CO_2], and water on morphospecies abundance (Table 5). Abundance was lower at each sample date in the elevated [CO_2] treatment, but no significant within-date treatment effects were observed (Fig. 16b). Water showed a less consistent trend, with abundance generally declining as the season progressed (Fig. 16a), resulting in a significant water x date interaction (Table 5). Abundance of morphospecies in the temperature treatment was unaffected across or within sample dates. Morphospecies richness significantly decreased under elevated temperature both across and within all sampling dates (Table 5; Fig. 17a). Although [CO_2] was significantly related to richness across dates, richness decreased under elevated [CO_2] only within May

Table 5 *P* – values and *df*[1] from repeated measures, mixed model ANOVA (Proc Mixed) for morphospecies abundance, richness, evenness, and H'. *P* – values in **bold** ≤ 0.10; n=12

Source	Abundance	Richness	Evenness	H'
CO_2	**0.047**	**0.055**	0.309	0.966
Temp	0.971	**<0.001**	0.123	**0.003**
Water	**0.029**	**0.098**	**0.075**	**0.049**
Date	**<0.001**	**<0.001**	0.102	**<0.001**
CO_2 x Temp	0.827	0.619	0.713	0.449
CO_2 x Water	0.749	0.787	0.843	0.298
Temp x Water	0.772	0.217	0.279	**0.029**
CO_2 x Date	0.282	**0.002**	**0.085**	**<0.001**
Temp x Date	**0.037**	**0.030**	0.586	0.218
Water x Date	**0.002**	**0.029**	0.749	0.627
CO_2 x Temp x Date	0.567	**0.015**	0.607	**0.015**
CO_2 x Water x Date	0.626	0.900	0.213	**0.031**
Temp x Water x Date	0.708	**0.073**	0.592	0.760
CO_2 x Temp x Water	0.581	**0.008**	0.373	**0.002**
CO_2 x Temp x Water x Date	0.348	**0.003**	0.930	**0.045**

[1] *df* =1,48 for CO_2, temp, water, CO_2 x temp, CO_2 x water, temp x water, CO_2 x temp x water. *df* =2, 48 for all other terms.

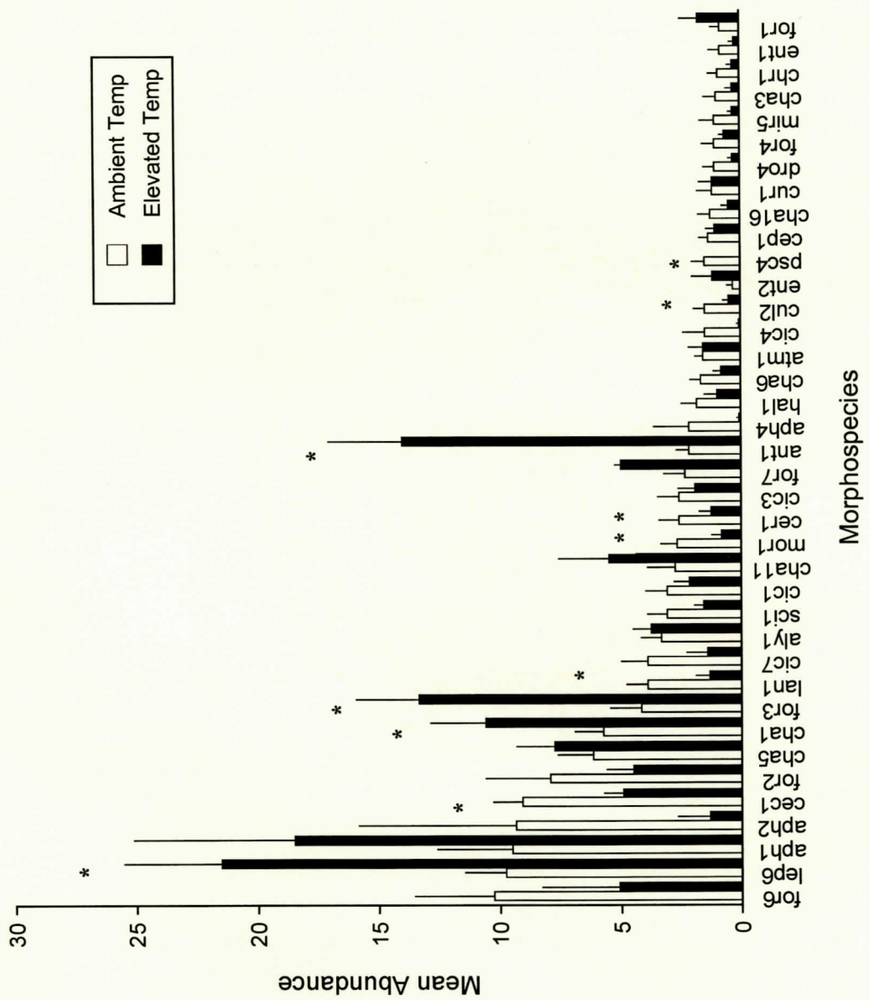

Fig. 15 Abundance (mean + 1 SE) for rank order of individual morphospecies at the ambient temperature (from most to least abundant) for ambient and elevated temperature. n=12; *= $p \leq 0.10$.

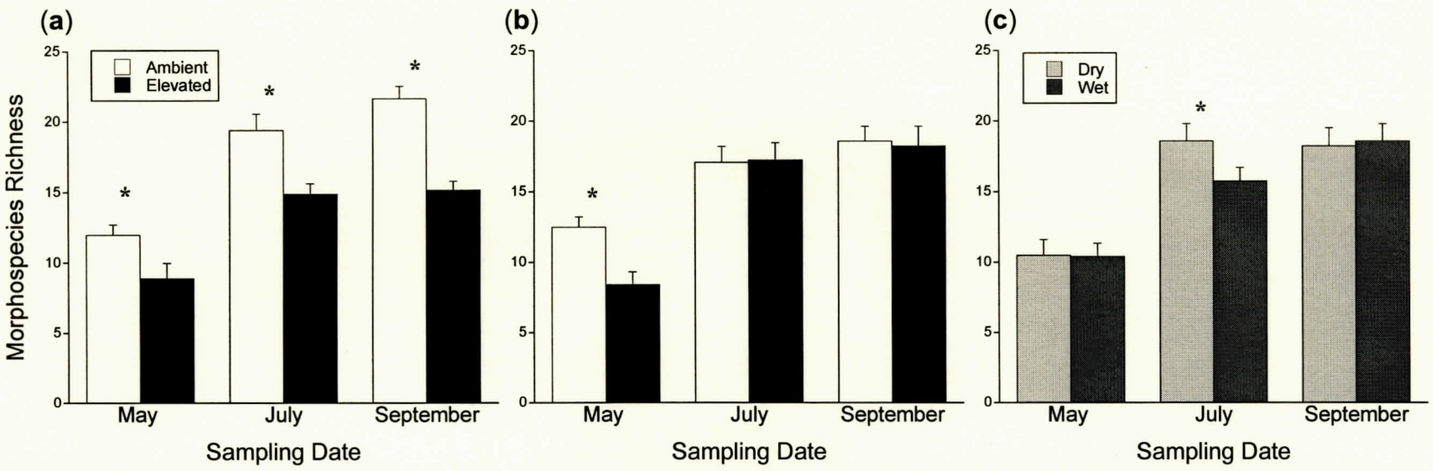

Fig. 17 Morphospecies richness (mean + 1 SE) by sampling date for (**a**) temperature, (**b**) carbon dioxide, and (**c**) water. n=12; *= $p \leq 0.10$. *P* – values generated by Proc Mixed within dates (SAS).

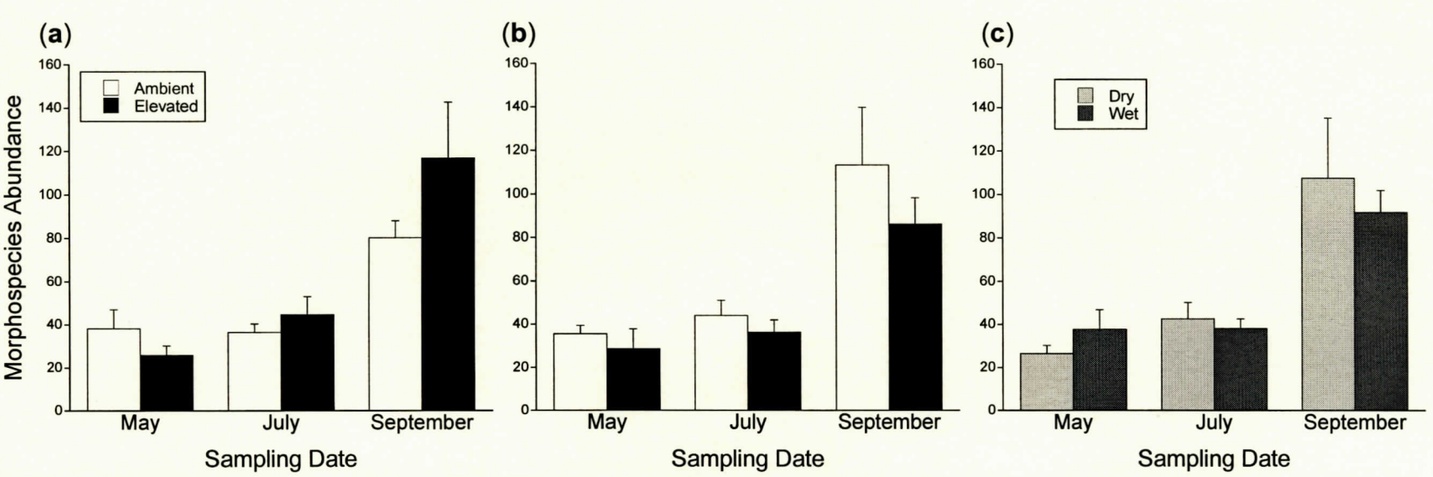

Fig. 16 Morphospecies abundance (mean + 1 SE) by sampling date for (**a**) temperature, (**b**) carbon dioxide, and (**c**) water. n=12.

(Table 5; Fig. 17b). The effect of temperature, [CO_2], and water on morphospecies richness all depended on date, resulting in significant temperature x date, CO_2 x date, and water x date interactions (Table 5). Evenness was related to water across all dates, but increased under the wet treatment only within July (Table 5; Fig. 17c). Morphospecies evenness was unaffected by temperature or [CO_2] (Fig. 18a-b), although there was a significant CO_2 x date interaction as evenness increased at elevated [CO_2] in the later sampling periods (Table 5; Fig 18b). Morphospecies Shannon-Wiener H' was significantly affected only by temperature across dates and within each date, with H' lower in the elevated temperature (Table 5; Fig. 19a). Treatment interactions of temperature x water and CO_2 x water were also observed (Table 5). Although Shannon-Wiener H' was unaffected by [CO_2] across dates, H' significantly decreased under elevated [CO_2] in May (Fig. 19b).

Plant-Insect Community Relationships

While rarefied predator richness was unrelated to rarefied herbivore richness (R^2 = 0.017, P = 0.543; Fig. 20a), rarefied parasitoid richness was positively correlated to rarefied herbivore richness (R^2 = 0.158, P = 0.054; Fig. 20b). The abundance of morphospecies was not related to key plant community parameters. Cumulative morphospecies abundance showed no relationship to ANPP or total plant community biomass (R^2 = 0.009, P = 0.669 and R^2 = 0.081, P = 0.177, respectively; Fig. 21a-b). Additionally, cumulative morphospecies rarefied richness also showed no relationship to ANPP or to total plant community biomass (R^2 = 0.086, P = 0.164 and R^2 = 0.062, P = 0.241, respectively; Fig. 22a-b).

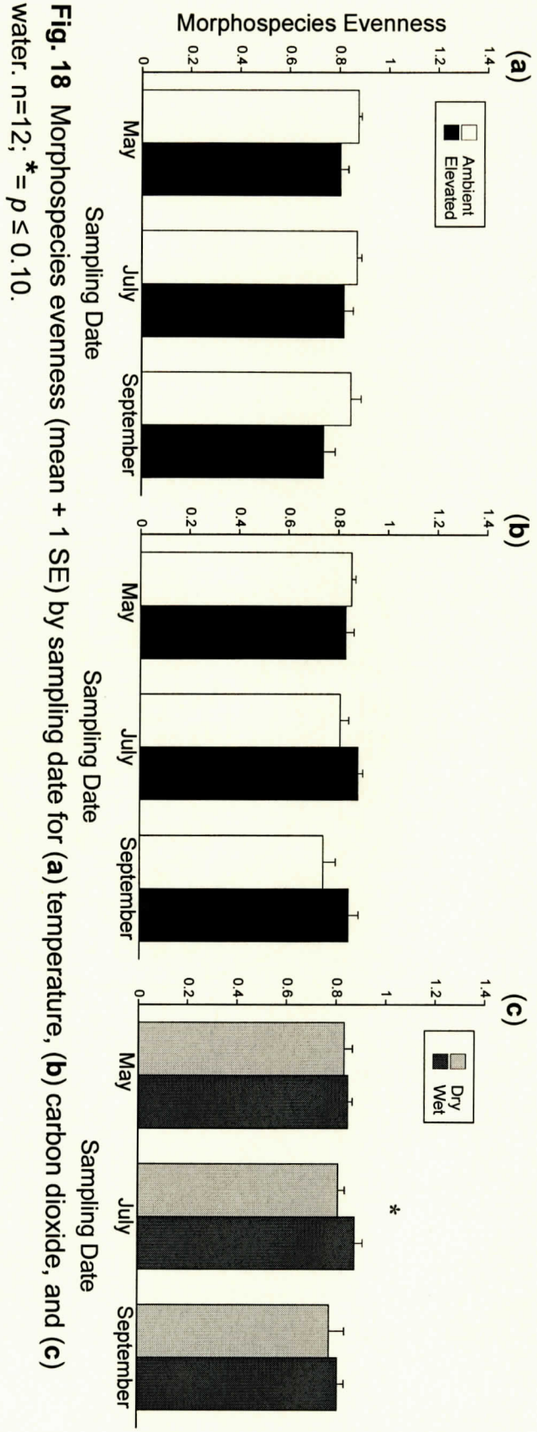

Fig. 18 Morphospecies evenness (mean + 1 SE) by sampling date for **(a)** temperature, **(b)** carbon dioxide, and **(c)** water. n=12; *= $p \leq 0.10$.

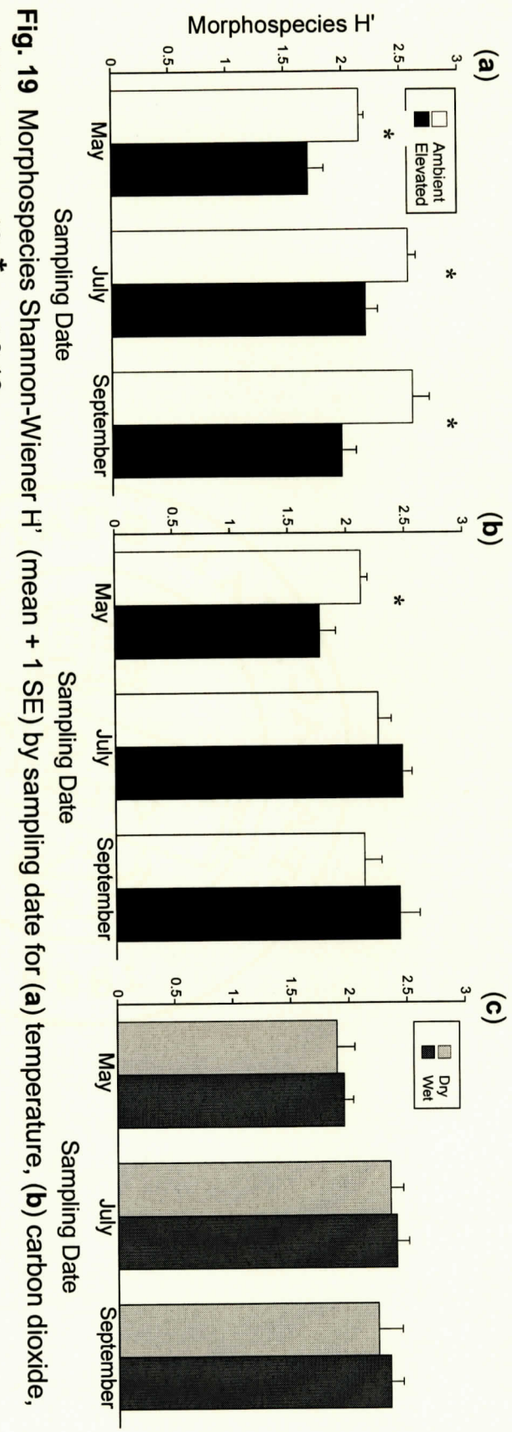

Fig. 19 Morphospecies Shannon-Wiener H' (mean + 1 SE) by sampling date for **(a)** temperature, **(b)** carbon dioxide, and **(c)** water. n=12; *= $p \leq 0.10$.

Rarefied richness for the dominant morphospecies at ambient and elevated temperature was unrelated to either ANPP or to total plant community biomass (R^2 = 0.045, P = 0.505 and R^2 = 0.110, P = 0.292, respectively; Fig. 23a-b).

Phytochemistry

Repeated measures ANOVA phytochemistry results are shown in Table 6 for *S. canadensis* and Table 7 for *L. cuneata*. For *S. canadensis*, all phytochemical constituents significantly changed over the season (Table 6). Briefly, [CO_2] significantly affected N content, while water significantly affected specific leaf weight, and water concentration in *S. canadensis* (Tables 6, 7). Temperature and water significantly interacted to affect *S. canadensis* N and C:N. In *L. cuneata*, all measures except water concentration changed over the season (Tables 8, 9). The [CO_2] treatment significantly affected N, C:N, water concentration, and specific leaf N. In this species temperature significantly affected N, specific leaf weight, and water concentration. Water significantly affected specific leaf weight and water concentration. For *L. cuneata* a large decline in leaf N early in the growing season (i.e. May) compared to late in the season (i.e. September) resulted in a significant CO_2 x date interaction (Table 8; Fig. 24c-d). For temperature, foliar C:N was more similar between ambient and elevated late in the season than early in the season (significant temp x date interaction, Table 8; Fig 25d).

Treatment effects on N and C:N within date were dependent upon plant species and collection date. For *S. canadensis*, elevated [CO_2] significantly decreased N content and significantly increased C:N in September ($F_{1,15}$ = 7.29, P =

0.017, and $F_{1,15}$ = 3.29, P = 0.090, respectively), but had no effect in May (P > 0.10; Fig. 24a-b). *Lespedeza cuneata* N content significantly decreased and C:N significantly increased under elevated [CO_2] in May ($F_{1,16}$ = 4.82, P = 0.044, and $F_{1,16}$ = 3.46, P = 0.081, respectively) and September ($F_{1,16}$ = 37.21, P = <0.001, and $F_{1,16}$ = 4.82, P = 0.044, respectively Fig. 24c-d).

Temperature had no effect on *S. canadensis* N content or C:N in either May or September (P > 0.10; Fig. 25a-b). For *L. cuneata*, elevated temperature significantly decreased N concentration and significantly increased C:N in May ($F_{1,16}$ = 7.69, P = 0.013, and $F_{1,16}$ = 7.56, P = 0.014, respectively), but had no effect in September (P > 0.10; Fig. 25c-d).

Water did not affect N content or C:N in *S. canadensis* (P > 0.10), while in *L. cuneata* leaf N concentration significantly increased and C:N significantly decreased in the wet treatment in May ($F_{1,16}$ = 7.07, P = 0.017, and $F_{1,16}$ = 8.07, P = 0.012, respectively), but had no effect in September (P > 0.10; Fig. 26c-d). Tannic acid equivalents were not affected by [CO_2], temperature or water treatments in *S. canadensis* (September only) or *L. cuneata* (May only) leaves (P > 0.10; Fig. 27).

Specific leaf weight, specific leaf N and water concentration are shown in Table 7 for *S. canadensis* and Table 9 for *L. cuneata*. *Solidago canadensis* specific leaf weight and water concentration were unaffected by [CO_2] or temperature (Table 7). Main effects of soil water increased leaf water concentration for *S. canadensis* by 6% in the dry treatment in September ($F_{1,16}$ = 3.49, P = 0.0825). There was a significant CO_2 x temperature interaction for specific leaf weight in May ($F_{1,16}$ = 4.13, P = 0.059). Leaf water concentration had a significant temperature x water

interaction ($F_{1,16}$ = 4.05, P = 0.063). *Solidago canadensis* specific leaf nitrogen was unaffected by [CO_2], temperature or water for May or September.

Lespedeza cuneata specific leaf weight increased by 24% in May and leaf water concentration decreased by 10% in May under elevated [CO_2] ($F_{1,16}$ = 11.23, P = 0.004, and $F_{1,16}$ = 12.96, P = 0.002, respectively; Table 9). Carbon dioxide concentration had no effect on either specific leaf weight or leaf water concentration in September. Elevated temperature increased *L. cuneata* specific leaf weight by 20% in September ($F_{1,16}$ = 7.90, P = 0.012; Table 9). Temperature did not affect *L. cuneata* leaf water concentration for May or September. Water decreased specific leaf weight by 12% in May ($F_{1,16}$ = 3.70, P = 0.073; Table 9), but had no effect on leaf water concentration.

Solidago canadensis specific leaf N was unaffected by [CO_2], temperature or water for May or September (P > 0.10). In *L. cuneata*, specific leaf nitrogen decreased by 30% under elevated [CO_2] and 35% under elevated temperature in May ($F_{1,16}$ = 11.59, P = 0.004 and $F_{1,16}$ = 13.32, P = 0.002, respectively; Table 9), with no effects in September.

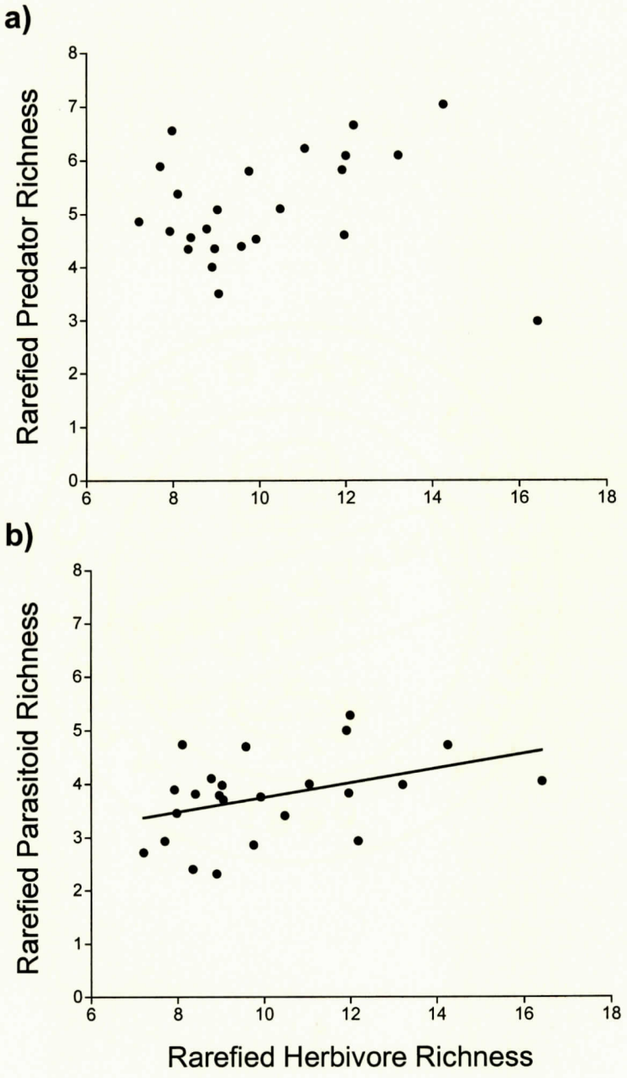

Fig. 20 Relationship between rarefied herbivore richness and (**a**) rarefied predator richness and (**b**) rarefied parasitoid richness for the dominant morphospecies dataset. n=24.

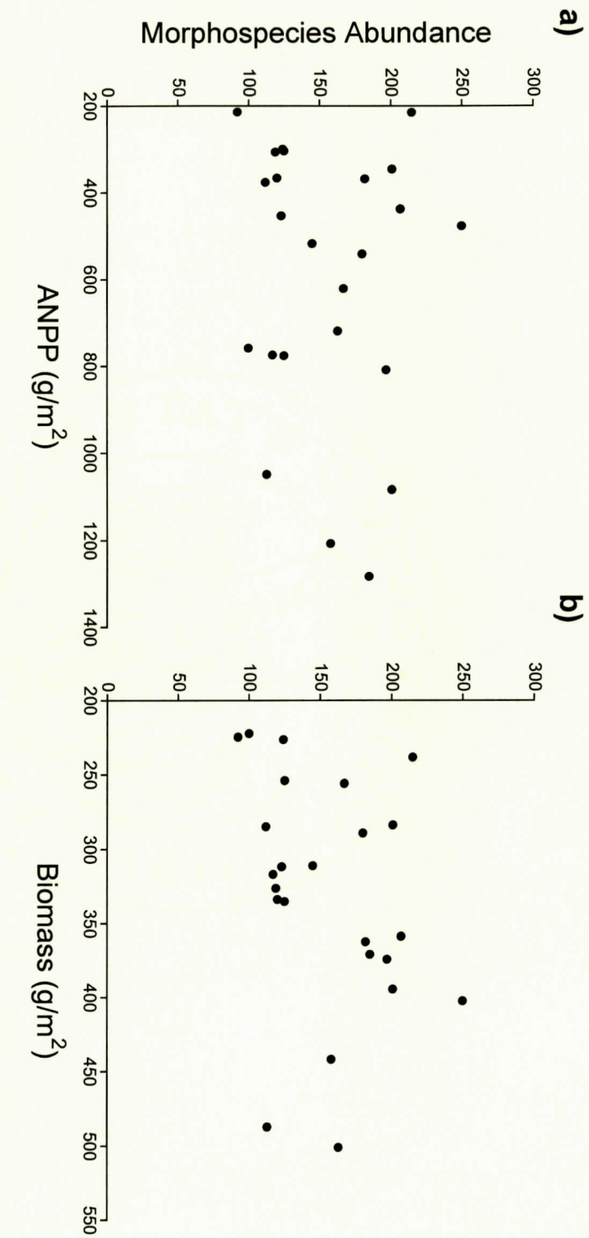

Fig. 21 Relationship between cumulative morphospecies abundance and (**a**) Above-Ground Net Primary Productivity (ANPP) and (**b**) total plant community biomass. n=24.

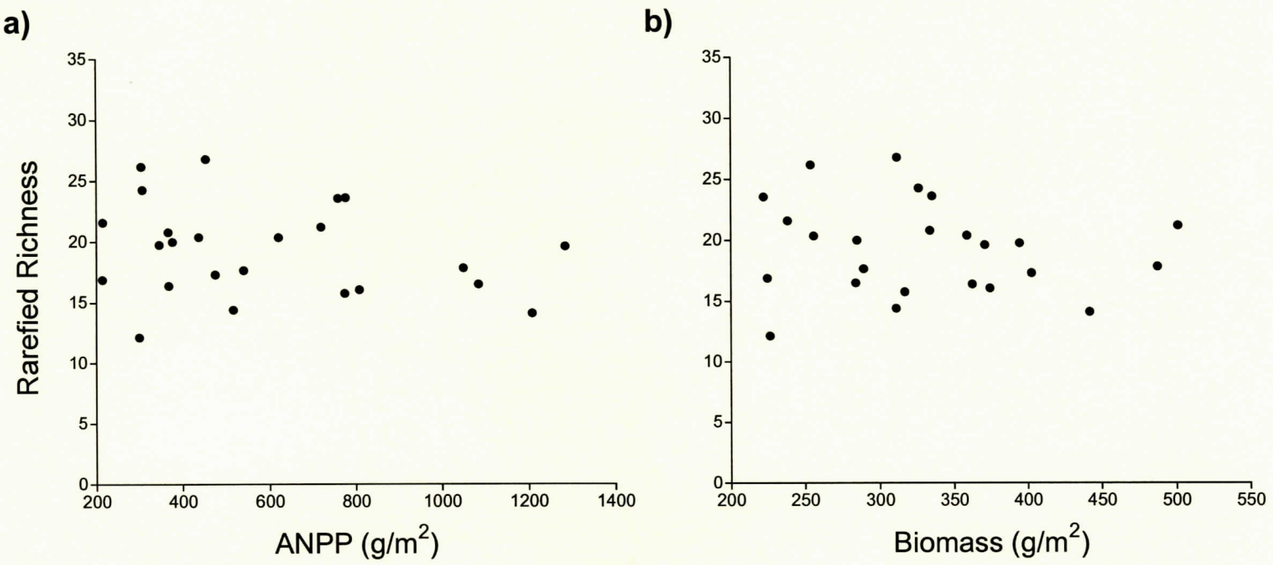

Fig. 23 Relationship between rarefied richness for the dominant morphospecies and (**a**) Above-Ground Net Primary Productivity (ANPP) and (**b**) total plant community biomass. n=24.

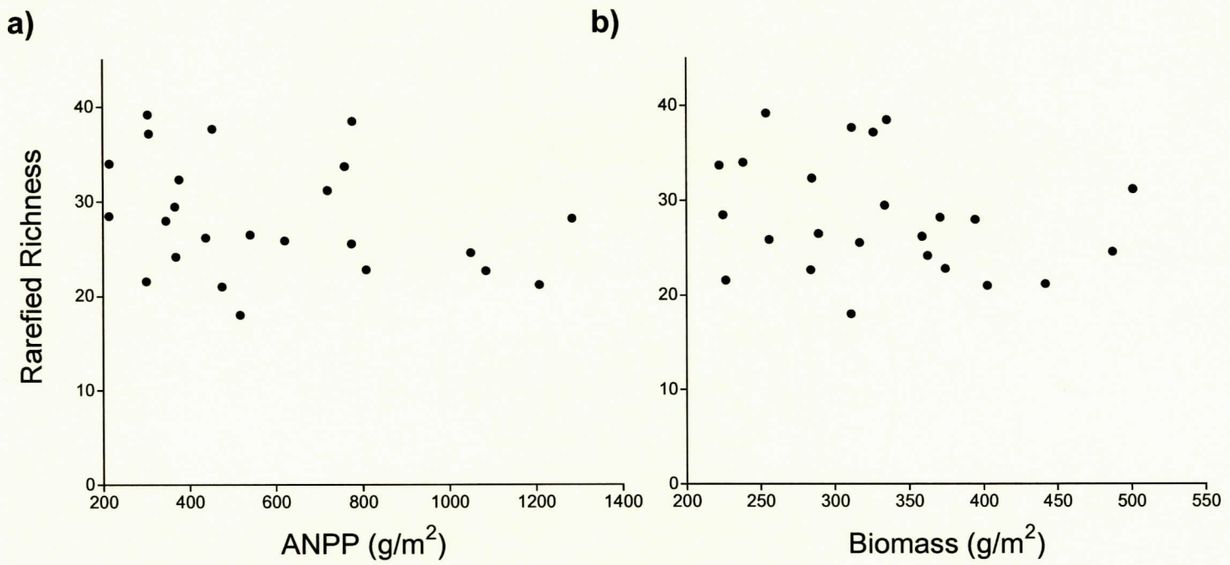

Fig. 22 Relationship between cumulative morphospecies rarefied richness and (**a**) Above-Ground Net Primary Productivity (ANPP) and (**b**) total plant community biomass. n=24.

Table 7 Leaf characteristics for *Solidago canadensis*, in May and September 2005 (mean ± 1 SE); n=12. Means significantly different within seasons ($P \leq 0.10$, Proc Mixed) are shown in **bold**

Leaf Characteristic	$[CO_2]$		Temp		Water	
	Ambient	Elevated	Ambient	Elevated	Wet	Dry
Specific Leaf Weight (g/cm^2)						
May	4.81 ± 0.33	5.50 ± 0.42	5.27 ± 0.42	5.02 ± 0.34	4.79 ± 0.21	5.52 ± 0.49
September	8.42 ± 0.65	8.65 ± 0.87	8.66 ± 0.91	8.40 ± 0.52	8.05 ± 0.70	9.10 ± 0.77
Specific Leaf N (mg/cm^2)						
May	2.29 ± 0.25	3.01 ± 0.50	2.40 ± 0.45	2.89 ± 0.30	2.27 ± 0.29	2.96 ± 0.45
September	0.81 ± 0.08	0.69 ± 0.07	0.70 ± 0.06	0.80 ± 0.09	0.78 ± 0.08	0.72 ± 0.07
Water Concentration (mg/g)						
May	770.4 ± 16.6	752.9 ± 18.0	746.7 ± 16.6	779.4 ± 17.1	783.0 ± 7.80	740.3 ± 21.7
September	681.7 ± 18.3	672.6 ± 19.8	685.6 ± 17.6	537.8 ± 8.40	**695.9** ± 19.5	**655.5** ± 15.4

Table 6 *P* – values and *df*[1] from repeated measures, mixed model ANOVA (Proc Mixed) for *Solidago canadensis* phytochemistry variables *P* – values in **bold** ≤ 0.10; n=12

Source	N	C:N	Specific Leaf Wt.	Water	Specific Leaf N
CO_2	**0.054**	0.177	0.255	0.533	0.930
Temp	0.962	0.581	0.890	0.969	0.335
Water	0.328	0.231	**0.081**	**0.036**	0.391
Date	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
CO_2 x Temp	0.349	0.806	0.509	0.568	0.500
CO_2 x Water	0.499	0.264	0.930	0.402	0.317
Temp x Water	**0.039**	**0.049**	0.170	0.113	0.421
CO_2 x Date	0.669	0.234	0.533	0.966	0.122
Temp x Date	0.737	0.875	0.831	0.159	0.650
Water x Date	0.726	0.586	0.771	0.974	0.224
CO_2 x Temp x Date	0.103	**0.038**	**0.082**	0.217	0.580
CO_2 x Water x Date	0.722	0.623	0.407	0.255	0.579
Temp x Water x Date	0.162	0.405	0.959	0.214	0.512
CO_2 x Temp x Water	0.742	0.786	0.512	0.768	0.855
CO_2 x Temp x Water x Date	0.761	0.421	0.215	0.466	0.768

[1] *df* =1,30 for all terms.

Table 9 Leaf characteristics for *Lespedeza cuneata*, in May and September 2005 (mean ± 1 SE); n=12. Means significantly different within seasons ($P \leq 0.10$, Proc Mixed) are shown in **bold**

Leaf Characteristic	[CO_2]		Temp		Water	
	Ambient	Elevated	Ambient	Elevated	Wet	Dry
Specific Leaf Weight (g/cm^2)						
May	**5.99** ± 0.34	**7.43** ± 0.30	6.52 ± 0.46	6.90 ± 0.30	**6.30** ± 0.34	**7.12** ± 0.39
September	9.72 ± 0.73	9.48 ± 0.45	**8.74** ± 0.49	**10.47** ± 0.61	**9.02** ± 0.50	**10.19** ± 0.66
Specific Leaf N (mg/cm^2)						
May	**4.10** ± 0.38	**2.86** ± 0.22	**4.20** ± 0.28	**2.75** ± 0.30	3.38 ± 0.32	3.58 ± 0.39
September	1.65 ± 0.27	1.41 ± 0.24	1.22 ± 0.20	1.84 ± 2.80	1.39 ± 0.25	1.68 ± 0.26
Water Concentration (mg/g)						
May	**588.4** ± 8.30	**528.9** ± 9.10	563.3 ± 13.4	554.0 ± 11.4	**569.8** ± 14.5	**547.5** ± 8.90
September	562.2 ± 16.4	541.6 ± 9.00	566.0 ± 16.2	537.8 ± 8.40	563.2 ± 13.1	540.6 ± 13.2

Table 8 *P* – values and *df*[1] from repeated measures, mixed model ANOVA (Proc Mixed) for *Lespedeza cuneata* phytochemistry variables *P* – values in **bold** ≤ 0.10; n=12

Source	N	C:N	Specific Leaf Wt.	Water	Specific Leaf N
CO_2	**<0.001**	**<0.001**	0.104	**0.004**	**0.090**
Temp	**0.005**	0.105	**0.026**	**0.081**	0.955
Water	0.154	0.238	**0.034**	**0.033**	0.259
Date	**<0.001**	**<0.001**	**<0.001**	0.485	**<0.001**
CO_2 x Temp	0.357	0.716	0.749	0.149	0.893
CO_2 x Water	0.460	0.450	0.503	0.568	0.498
Temp x Water	0.878	0.489	0.814	0.973	0.863
CO_2 x Date	**0.005**	**0.045**	**0.047**	**0.049**	0.585
Temp x Date	**<0.001**	**0.015**	0.342	0.375	**<0.001**
Water x Date	0.228	0.499	0.967	0.916	0.351
CO_2 x Temp x Date	0.532	0.349	0.881	**0.041**	0.676
CO_2 x Water x Date	0.788	0.171	0.385	0.593	0.887
Temp x Water x Date	0.565	0.786	0.170	0.245	0.611
CO_2 x Temp x Water	0.476	0.548	0.361	0.898	0.899
CO_2 x Temp x Water x Date	0.356	0.299	0.936	0.556	0.943

[1] *df* =1,32 for all terms.

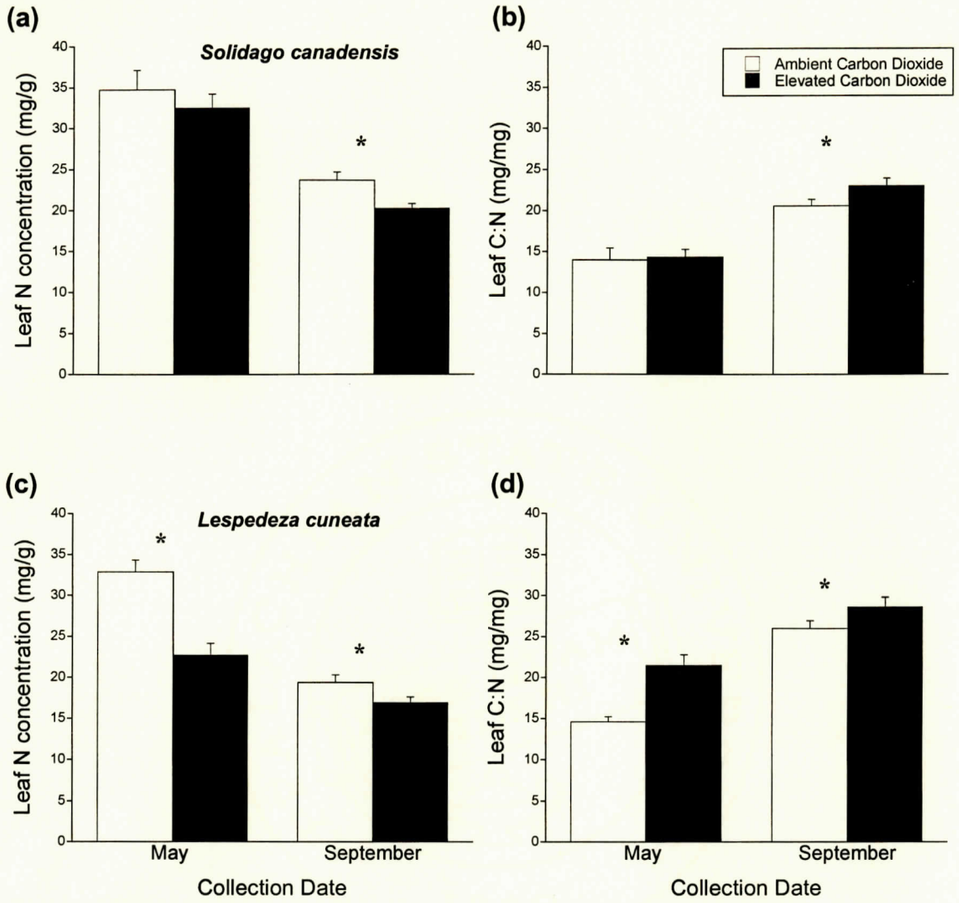

Fig. 24 Effects of [CO_2] (mean + 1 SE) on foliar N (**a, c**) and C:N (**b, d**) for *Solidago canadensis* (top panels) and *Lespedeza cuneata* (bottom panels). n=12; *= $p \leq 0.10$. *P* – values generated by Proc Mixed within species and date (SAS).

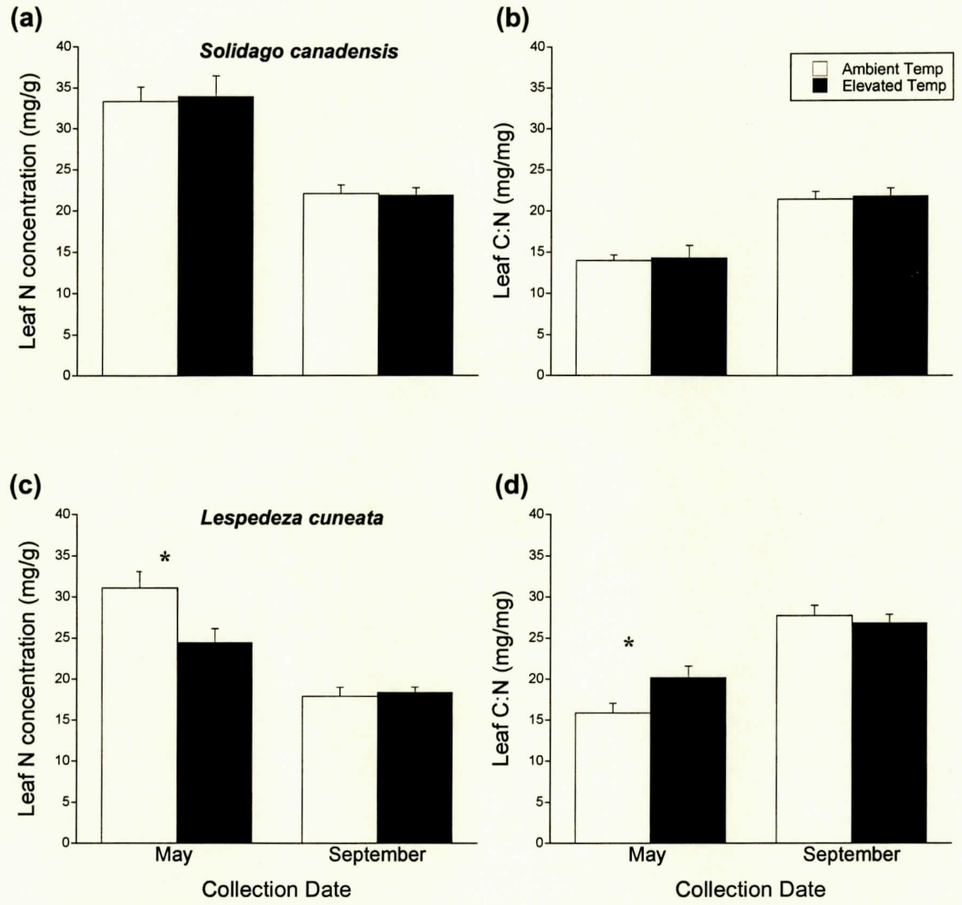

Fig. 25 Effects of temp (mean + 1 SE) on foliar N (**a, c**) and C:N (**b, d**) for *Solidago canadensis* (top panels) and *Lespedeza cuneata* (bottom panels). n=12; *= $p \leq 0.10$.

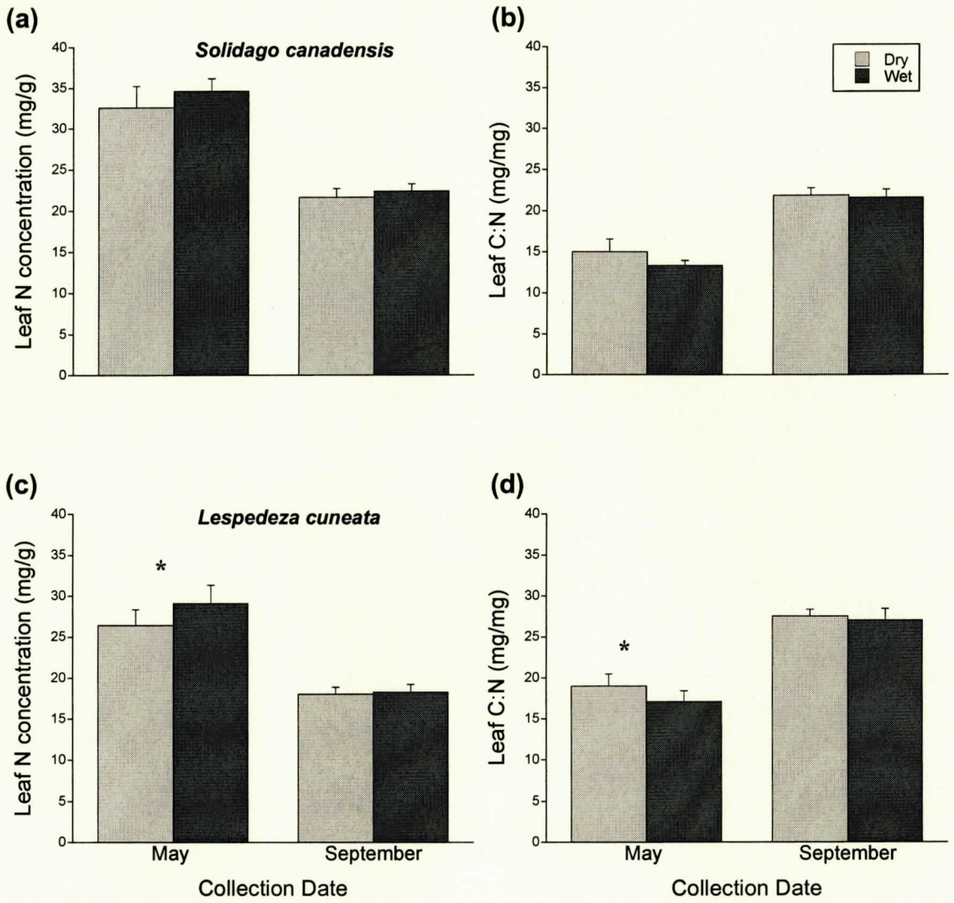

Fig. 26 Effects of water (mean + 1 SE) on foliar N (**a, c**) and C:N (**b, d**) for *Solidago canadensis* (top panels) and *Lespedeza cuneata* (bottom panels). n=12; *= $p \leq 0.10$.

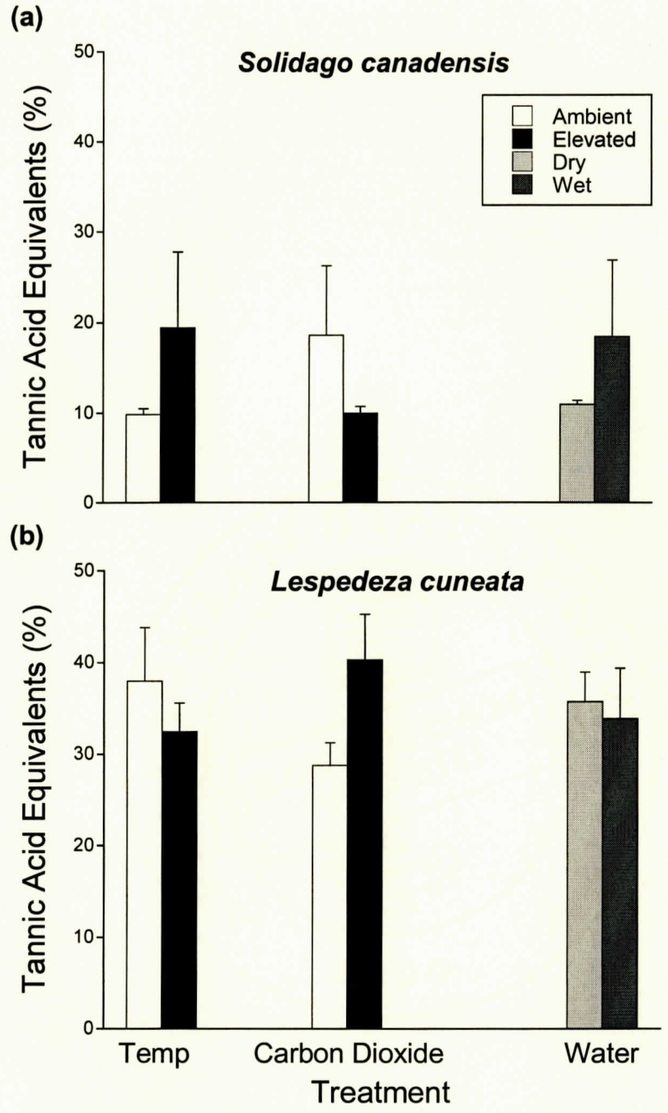

Fig. 27 Tannic acid equivalents (mean + 1 SE) for (**a**) *Solidago canadensis* (September only) and (**b**) *Lespedeza cuneata* (May only) leaves for temperature, carbon dioxide, and water. n=12.

DISCUSSION

The structure of plant and insect communities is important to ecosystem function. Thus, understanding how predicted climate change may affect plant-insect community associations is crucial for predicting how large scale terrestrial ecosystems may change in the future. My research represents the first study on insect community structure within a plant community exposed to simultaneous [CO_2], temperature, and water manipulation. My results show that temperature, more so than [CO_2] or water, is responsible for changing insect community composition via effects on diversity and feeding guild composition. The community-level approach in my study, in concert with demonstrated changes in insect community parameters due to a principal climate driver, addresses a need for data allowing broader predictability of how multiple climate change factors may alter important terrestrial ecosystems.

I set out in my study to address four questions allowing me to examine how an old-field insect community could be changed by the simultaneous application of three important climate drivers. First, I asked whether application of these factors would affect insect community composition. I predicted that within one growing season an elevated temperature would directly affect the insect community by increasing total morphospecies abundance, overall diversity and diversity at the level of feeding guilds. This approach allowed me to evaluate community effects in both the insect community as a whole and in functional groups. Sticky trap data did reveal differences in insect abundance due to temperature, [CO_2], and water.

Caution should be used in interpreting these results for water, since the wet and dry subplots were in close proximity within chambers, potentially affecting the accuracy of trapping actively flying insects.

Thysanoptera (thrips), whose members are important herbivores and often economic pests, increased in abundance by 122% under the elevated temperature treatment (Fig. 3). Observed treatment effects in this insect Order may be especially relevant. Edelson & Magaro (1988) demonstrated that reproduction and development of a common thrips were strongly related to temperature. Analysis of climate change models and predicted changes in temperature show that the number of generations per year in thrips is likely to increase as temperatures rise (Bergant *et al.*, 2005). In agreement with these previous studies, my study demonstrates that temperature affects this particular Order. In addition to temperature, I found a positive increase in the abundance of other insect Orders (i.e. Homoptera and Diptera) with sufficient soil water availability. While my insect abundance data from sticky traps suggest that insect Orders may be affected by future climate change, caution is needed in interpreting these results. This is because sticky traps are attractive to some insects and not others (Hoback *et al.*, 1999), therefore differentially sampling insects. Because very small insects may be underrepresented using other sampling techniques (e.g. vacuuming), I used the sticky trap data primarily to augment my vacuum sample data. Despite some limitations, the strong effect of temperature on the abundance of a significant herbivore Order using traps provides clear evidence of the direct effect of an important climate driver (i.e. temperature). As expected, this result was especially

relevant because due to their small size, thrips were indeed underrepresented in the vacuum samples.

Cumulative insect abundance in vacuum samples did not differ in response to any treatment in this study (Table 3), in contrast to my original prediction. My study did, however, find a significant increase in predator, and decrease in detritivore, abundance due to elevated temperature (Table 3; Fig 6a). In a study examining the insect community of a plant understory system grown under elevated [CO_2], Sanders *et al.* (2004) also found no difference in total arthropod abundance. Other climate change studies with one climate driver ([CO_2]) have shown lower herbivore density in forest systems grown under elevated [CO_2] (Stiling *et al.*, 2002, 2003; Hamilton *et al.*, 2004), thus community-level effects of a single climate driver are known. Comparisons between my old-field community study and forest are difficult due to the potentially large effect that plant species composition has on insect communities. With respect to temperature, studies have shown positive relationships between insect abundance (Whittaker & Tribe, 1998) and richness (Andrew & Hughes, 2005). Richardson *et al.* (2002), however, found a decrease in abundance of a dominant herbivore under elevated temperature treatments. One possible reason for a decrease in abundance is that even though insects often develop faster at elevated temperature, adults are shown to have lower weight and fecundity (Bale *et al.*, 2002). This means that the beneficial results of elevated temperature in the short term would not carry over to the population level, nor would such beneficial results persist across generations. Based on previous studies and the temperature effects on insect community diversity measures observed in my

study (see below), future studies in the model old-field plant-insect community I investigated should measure temperature effects on insect fecundity.

Because of a possible link with abundance I predicted that an elevated temperature would increase morphospecies diversity both within and across feeding guilds. My data, however, demonstrate that richness, evenness, and Shannon-Wiener H' actually decreased under elevated temperature, while neither elevated [CO_2] nor water treatments had any effect upon diversity measures (Table 3; Fig. 5). Also relevant is a lack of interactions between my three climate factors and insect diversity measures. Therefore, my study strongly demonstrates that temperature alone is the most important factor explaining variation in the cumulative insect community diversity parameters within this model old-field community. Further, when I account for differences in abundance between subplots using rarefaction, species richness is unrelated to temperature ($P = 0.110$), demonstrating that the observed effect on richness at elevated temperature is due to individuals. Effects of increasing abundance on richness are predicted from previous work (e.g. Srivastava & Lawton, 1998), and while I did not find significantly greater abundance of insects at the elevated temperature, the data trended this way, making a decrease in richness unexpected. Along with observed effects on morphospecies richness, the lower evenness at elevated temperature demonstrated a potential shift to more dominant morphospecies, since evenness is a value ranging from 0 (all abundance in one species) to 1 (all species are in equal abundance; Schmitz, 2003; Mulder *et al.*, 2004). Similarly, lower Shannon-Wiener H' at the elevated

temperature suggests community-level shifts due to a single driver, potentially due to the direct effects of temperature on some species compared to others.

Using a non-metric multidimensional scaling ordination to analyze my cumulative morphospecies data for temperature, [CO_2], and water shows that temperature alone had an effect on community structure (Figs. 10-13). This analysis assesses the dimensionality of community data by examining the overall dataset compared to a main treatment factor (i.e. [CO_2], temperature or water; McCune & Mefford, 1999). Community similarity based on treatments (calculated as an abundance-based Sørensen index) also showed that the communities under ambient versus elevated temperature are more dissimilar than those under ambient versus elevated [CO_2], or dry versus wet treatments (Table 4). These analyses both clearly support a shift in insect community composition (primarily based on richness) due to temperature, but not [CO_2], or water.

Even though I observed no significant effect of temperature on insect abundance within one growing season, it was relevant to focus further on this individual factor because temperature alone affected other insect diversity parameters (i.e. richness, evenness, and H'; Table 3). An examination of the data found that 38 morphospecies accounted for over 90% of the abundance at the ambient temperature. In this group, 10 species increased in abundance under elevated temperature, while 28 decreased (Figs. 13-15). Calculating the percent change in abundance at elevated compared to ambient temperature showed that a few morphospecies increased dramatically in abundance. This could help explain the increase in abundance at elevated temperature (non-significant). From this

dominant species data set I conclude that while overall there were no treatment effects on cumulative abundance of morphospecies in my study, clearly temperature did have both positive and negative impacts on a number of the most abundant morphospecies collected.

In addition to abundance, the dominant morphospecies data based on ambient and elevated temperature provided insight into treatment effects on diversity. Of the 38 dominant species (again, representing over 90% of the total abundance in my experiment), only two species were not found at elevated temperature (Table 4; Fig. 13). The high Sørensen similarity index of morphospecies (98.8) between ambient and elevated temperature supports the conclusion that there was little effect of temperature on reducing morphospecies richness for the dominants, helping to explain the observed reductions in richness with all species considered (Fig. 5a). What appears to be driving the reduction in species richness at elevated temperature is the absence of species (total identified; 104 at elevated temperature and 130 at ambient temperature) found in very low abundance (Table 4).

Analysis of guild data also revealed significant reductions in richness and Shannon-Wiener H' for herbivores, detritivores, and parasitoids under elevated temperature, while no or limited effects due to [CO_2] and water were observed. Unlike the cumulative dataset (all guilds combined) treatment effects on guild abundance were observed in certain guilds. While herbivore abundance was higher at elevated temperature (though non-significant), predator abundance increased and detritivores declined (Fig. 6a). The only main treatment effect other than

temperature was an increase in parasitoids under dry soil conditions. Generally, the guild data are consistent with the cumulative morphospecies data, in that temperature is the driver that is most influencing guild structure within this insect community. Climate drivers largely did not interact to affect guild responses (Table 3). Predator richness, however, was not significantly reduced (in contrast to the other three guilds) possibly because the magnitude of the temperature effect was different for high and low [CO_2] (significant CO_2 x temp interaction). Detritivores did show a significant reduction in richness at elevated temperature, but again the magnitude of that change was dependent on [CO_2] level, resulting in a significant CO_2 x temp interaction in this guild as well (Table 3; data not shown). Sanders *et al.* (2004) examined trophic structure of an understory arthropod community and similarly found no effect of elevated [CO_2] on herbivore, detritivore, predator or parasitoid richness. My community-level results show strong similarities with this previous study that focused on [CO_2], although I conclude that temperature is the principal climate driver in my model old-field community system.

Since trophic levels of insect communities are often correlated (Siemann *et al.*, 1998; Koricheva *et al.*, 2000), I predicted that changes in one insect trophic level would be reflected in higher trophic levels (see Siemann *et al.*, 1998; Knops *et al.*, 1999). For example, my study observed that effects on herbivores could contribute to treatment-level responses in higher trophic levels, though the trajectory of the response could differ within guilds if they were differentially affected by climate drivers. Previous studies provide evidence that changes in one trophic level results in a response within another in terrestrial ecosystems. For example, parasitoid

richness has been shown to positively correlate with herbivore richness in relatively simple host plant – leaf miner – parasitoid systems (Kruess, 2003) and strong relationships between herbivores and predators/parasitoids are known (e.g. Siemann *et al.*, 1998; Knops *et al.*, 1999). In my study, predator abundance increased at elevated temperature along with herbivore abundance, though this response was non-significant (Fig. 6a). Similarly, diversity measures affected by temperature corresponded between guilds (e.g. reduced herbivore, predator and parasitoid richness and H' at elevated temperature, Figs. 7a, 9a). Finally, linear regression demonstrated a significant (although weak) relationship between rarefied herbivore and parasitoid richness (Fig. 20b). This particular relationship could be sensitive to climate change because studies have found that reductions in host plant quality, which I partially demonstrated in my study, indirectly reduced the fitness of insect parasitoids (Francis *et al.*, 2001). I conclude that even though specific insect species-species interactions are not yet elucidated in this old-field community, my data demonstrate that trophic level interactions are being altered due to an important predicted climate change driver (i.e. temperature).

I predicted at the outset of my study that [CO_2]-induced alterations in plant quality would impact the insect community, especially herbivores. While I found significant decreases in N and increases in C:N in two dominant host plants due to [CO_2] (Fig. 24), no effects of [CO_2] on either cumulative morphospecies diversity or diversity for herbivores or any other guild were observed. Phytochemical changes due to elevated [CO_2] have been shown to increase mortality (Coviella & Trumble, 1999), increase natural enemy abundance (Percy *et al.*, 2002) and decrease insect

fecundity (Awmack &Leather, 2002), any of which can negatively affect insect herbivore populations. However, my data are not supportive of my prediction that reductions in foliar quality will impact the herbivore guild. The lack of a [CO_2] effect on this guild strongly suggests that herbivores within this intact system were able to overcome observed decreases in plant nutritional quality, perhaps by consuming more tissue to satisfy N requirements or by increasing N use efficiency as many studies have found in other systems (e.g. Lincoln *et al.*, 1993; Williams *et al.*, 1998).

Previous climate change studies have found limited effects of temperature on phytochemistry in tree species (Williams *et al.*, 2000, 2003), while in this study an elevated temperature reduced the nutritional quality (lower N and higher C:N) in one plant species I investigated, *Lespedeza cuneata* (Fig. 25c-d). My observed reduced morphospecies richness and H' early in the growing season (Figs. 17a, 19a), when temperature effects on *L. cuneata* quality were observed, suggest the potential for phytochemically-mediated effects by temperature on insect community parameters. Because I focused on a limited number of plant species (i.e. 2 of 7 total in the community) for a phytochemical assessment, my study was unable to fully develop effects of leaf phytochemistry on insect community parameters. *Solidago canadensis* did show a significant reduction in foliar N under elevated [CO_2] but no effects of temperature or water (Table 6). However, an effect of water is evident, since the slight temperature effect on *S. canadensis* leaf N depended on water treatment (i.e. elevated temperature reduced N under the dry treatment and increased it under the wet treatment), which led to a significant temp x water interaction (Table 6; data not shown). The reduction in leaf nutritional quality in *L.*

cuneata at both [CO_2] and temperature, with concurrent reductions in important insect diversity measures, suggests that treatment effects on leaf nutritional chemistry may be acting on the insect community in my study. Finally, the lack of data on herbivory levels does not allow me to closely link phytochemical alterations with observed insect trophic level responses. Herbivory should be closely monitored in future studies of this system to see if insects are indeed consuming more tissue in cases where a reduction in nutritional quality is demonstrated.

Because both plant and insect community characteristics are known to change over a growing season (Scheirs *et al.*, 2002; Kaneko, 2005) I asked if treatment effects in the insect community were seasonally dependent. A repeated measures ANOVA demonstrated main effects of [CO_2], water, and interactions on abundance, richness, evenness, and H' (Table 5). For all measures observed, values increased with the season, irrespective of treatment. While the consideration of all sample dates does demonstrate treatment effects not observed in the cumulative analysis (e.g. [CO_2], water, and interactions) the within date analysis is mostly in agreement with effects in the entire growing season. Abundance was not significantly related to treatment on any collection date, although temperature and water both interacted with date for abundance, i.e., the temperature effect on abundance was opposite and differed in magnitude at different sampling dates (Table 5). Both morphospecies richness and Shannon-Wiener H' significantly declined at elevated temperature in every sample (Figs. 17, 19). A decline in richness and H' in the elevated [CO_2] and temperature treatment in May (Figs. 17, 19) suggests that the observed reduction in plant nutritional quality (i.e. reduced leaf

N and increased leaf C:N, *Lespedeza cuneata*, Fig. 24) could affect diversity-level parameters within a portion of the growing season. It is known that elevated [CO_2] can affect insect communities via changes in plant nutritional quality (Stiling *et al.*, 2002, 2003; Knepp *et al.*, 2005), though effects of elevated temperature on intact insect communities via phytochemical alterations are less clear.

Because the effects of climate drivers in the overall OCCAM experiment are directed toward the plant community responses, I asked whether the insect community was related to plant community primary productivity and biomass. Abundance and diversity of resources should support a diversity of species at higher trophic levels (Whittaker, 1975; Tilman, 1986; Rosenweig, 1995). Therefore, more productive or diverse plant communities should support a more abundant and diverse insect community. For example, past studies have shown a positive correlation between plant species richness and insect guild (herbivore and predator) richness (Siemann *et al.*, 1998; Knops *et al.*, 1999). In 2005, plant community richness was unaffected by temperature or [CO_2] within the OCCAM experiment (Engel *et al.*, unpublished data). My data on the insect community demonstrated no relationship between ANPP or biomass and cumulative morphospecies abundance, cumulative morphospecies rarefied richness, or dominant morphospecies rarefied richness (Figs. 21-23). Because my study did not set out to sample insect community parameters and plant productivity simultaneously, producing reliable relationships on a cumulative basis is challenging. Clearly, a link between the plant and insect community and potential effects of these climate drivers on their association is needed in future studies.

Conclusion

This study demonstrates that an old-field insect community exposed to simultaneous [CO_2], temperature, and water treatments is primarily affected by temperature, with few interactions at the cumulative morphospecies level. An elevated temperature altered the insect community via reductions in richness, evenness, and Shannon-Wiener H' within a single growing season. Reductions in richness are best explained by the absence of morphospecies found at very low abundance, as evidenced by my dominant morphospecies data. In addition, decreases in richness and Shannon-Wiener H' were observed within feeding guilds due to an elevated temperature, with evidence of herbivores affecting the richness and diversity of higher order trophic levels (i.e. predators and parasitoids). My conclusions on temperature effects are supported by several analyses. Non-metric multidimensional scaling demonstrated that temperature alone is affecting morphospecies richness and community composition and a modified Sørensen similarity index showed communities at ambient versus elevated temperature shared less similarity than for [CO_2] or water treatments. A decline in cumulative morphospecies evenness, along with reduced evenness of the dominant morphospecies due to temperature, suggest this climate driver is shifting the dominance of some insect species within this old-field community. My results are important in the context of climate change research because this system consists of intact plant and insect communities that are receiving multiple, potentially interacting climate change factors. Since these conditions represent a more accurate simulation of future climate conditions (i.e. intact systems under multiple factors) my

data give a more realistic prediction of how insect communities may change as global climate changes, and provide a framework for future, more directed investigations within this ecosystem.

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APPENDIX

List of Identified Morphospecies and Corresponding Guilds

Diptera		
Anthomyzidae	Atm1	Detritivore
Asteiidae	ast1	Detritivore
Cecidomyiidae	cec1	Herbivore
	cec2	Herbivore
	cec3	Herbivore
Ceratopogonidae	cer1	Predator
	cer2	Predator
Drosophilidae	dro1	Detritivore
	dro2	Detritivore
	dro3	Detritivore
	dro4	Detritivore
	dro5	Detritivore
Muscidae	mus1	Detritivore
Otitidae	oti1	Detritivore
Psycodidae	psy1	Detritivore
Sciaridae	sci1	Detritivore
	sci2	Detritivore
Sphaeroceridae	sph1	Detritivore
Syrphidae	syr1	Herbivore
Tipulidae	tip1	Detritivore

Coleoptera		
Chrysomelidae	chr1	Herbivore
	chr2	Herbivore
	chr3	Herbivore
	chr4	Herbivore
	chr5	Herbivore
Coccinelidae	coc1	Predator
	coc2	Predator
	coc3	Predator
Culicidae	cul1	Detritivore
	cul2	Detritivore
Curculionidae	cur1	Herbivore
	cur2	Herbivore
	cur3	Herbivore
	cur4	Herbivore
	cur5	Herbivore
	cur6	Herbivore
	cur7	Herbivore
	cur8	Herbivore
	cur9	Herbivore
Lathridiidae	lan1	Detritivore
Mordellidae	mor1	Herbivore
	mor2	Herbivore
Phalacridae	pha1	Herbivore
Scarabaeidae	sca1	Herbivore

Homoptera		
Acanaloniidae	ana1	Herbivore
Aphididae	aph1	Herbivore
	aph2	Herbivore
	aph3	Herbivore
	aph4	Herbivore
Cercopidae	cep1	Herbivore
	cep2	Herbivore
Cicadellidae	cic1	Herbivore
	cic2	Herbivore
	cic3	Herbivore
	cic4	Herbivore
	cic5	Herbivore
	cic6	Herbivore
	cic7	Herbivore
	cic8	Herbivore
	cic9	Herbivore
	cic10	Herbivore
	cic11	Herbivore
	cic12	Herbivore
	cic13	Herbivore
	cic14	Herbivore
	cic15	Herbivore
Issidae	iss1	Herbivore
	iss2	Herbivore

Collembola		
Entomobryidae	ent1	Detritivore
	ent2	Detritivore

Pscoptera		
	psc1	Detritivore
	psc2	Detritivore
	psc3	Detritivore
	psc4	Detritivore

Lepidoptera		
Ctenuchidae	cte1	Herbivore
Lepidoptera	lep2	Herbivore
	lep3	Herbivore
	lep4	Herbivore
	lep5	Herbivore
	lep6	Herbivore
	lep7	Herbivore
Lycaenidae	lyc1	Herbivore
Pterophoridae	pte1	Herbivore

Hymenoptera		
Brachonidae	bra1	Parasitoid
	bra2	Parasitoid
	bra3	Parasitoid
	bra4	Parasitoid
Chalcidoidae	cha1	Parasitoid
	cha2	Parasitoid
	cha3	Parasitoid
	cha4	Parasitoid
	cha5	Parasitoid
	cha6	Parasitoid
	cha7	Parasitoid
	cha8	Parasitoid
	cha9	Parasitoid
	cha10	Parasitoid
	cha11	Parasitoid
	cha12	Parasitoid
	cha13	Parasitoid
	cha14	Parasitoid
	cha15	Parasitoid
	cha16	Parasitoid
	cha17	Parasitoid
	cha18	Parasitoid
	cha19	Parasitoid
	cha20	Parasitoid
	cha21	Parasitoid
	cha22	Parasitoid
	cha23	Parasitoid
	cha24	Parasitoid
	cha25	Parasitoid
Colletidae	col1	Herbivore
	col2	Herbivore
Formicidae	for1	Predator
	for2	Predator
	for3	Predator
	for4	Predator
	for5	Predator
	for6	Predator
	for7	Predator
Halictidae	hal1	Herbivore
	hal2	Herbivore
	hal3	Herbivore
	hal4	Herbivore
Vespidae	ves1	Predator
	ves2	Predator

Thysanoptera		
Thripidae	thr1	Herbivore

Hemiptera		
Anthocoridae	ant1	Predator
	ant2	Predator
Alydidae	aly1	Herbivore
Berytidae	ber1	Herbivore
Lygaeidae	lyg1	Herbivore
	lyg2	Predator
Miridae	mir1	Predator
	mir2	Herbivore
	mir3	Herbivore
	mir4	Herbivore
	mir5	Herbivore
	mir6	Herbivore
	mir7	Herbivore
Nabidae	nab1	Predator
	nab2	Predator
	nab3	Predator
Pentatomidae	pen1	Herbivore
	pen2	Herbivore
Reduviidae	red1	Predator
Thyreocoridae	thy1	Herbivore
Tingidae	tin1	Herbivore

BIOGRAPHICAL SKETCH

Shawn Nathan Villalpando was born on January 23, 1981 in Santa Barbara, California. Shawn attended Central Piedmont Community College in Charlotte, North Carolina for three years (1999-2001), and graduated with an Associate of Science degree. In spring 2002, he enrolled at the University of North Carolina at Charlotte, and earned a Bachelor of Science with an option in Ecology degree in May 2004. Shawn then relocated to Boone, North Carolina and entered the Biology graduate program at Appalachian State University in August 2004. Shawn graduated with his Master of Science degree in Biology in May 2007.