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Can atmospheric composition influence plant fossil preservation potential via changes in leaf mass per area? A new hypothesis based on simulated palaeoatmosphere experiments.

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ABSTRACT

Atmospheric composition, particularly levels of CO_2 and O_2, impacts all aspects of life but its role in relation to plant preservation in the fossil record is largely unconsidered. Plants, angiosperms in particular, have been widely shown to increase leaf mass per area (LMA) under high CO_2 conditions and decrease LMA in low CO_2 conditions. Leaf thickness has long been known to be a contributory factor in preservation potential in the plant fossil record, with thicker leaves considered to have a greater recalcitrance than thinner ones. Therefore, any change in leaf density/thickness, through changes to LMA, could lead to an increased or decreased preservation potential of fossil leaves at times of elevated or decreased CO_2, respectively. . Additionally, the impact of changes to atmospheric O_2 and to the atmospheric CO_2:O_2 ratio on LMA has not been previously considered in detail. This investigation examines the effect of simulated Mesozoic atmospheres, times of high CO_2 and low O_2, on LMA in a suite of gymnosperms that act as nearest living equivalents for common elements of Mesozoic floras. Exposure to high CO_2 (~1,500 ppm) led to a statistically significant ($p < 0.001$) increase in LMA in four out of 6 species, and exposure to combined high CO_2 and low O_2 (~13%) induced a statistically significant ($p < 0.001$) increase in LMA in all six species. The investigation also examined the effects of atmospheric composition on %N, a key plant trait known to co-vary with LMA under modern atmospheric compositions that provides information on plant function and relates to photosynthetic efficiency. Most species showed decreased %N in treatments with increased LMA in agreement with modern ecological studies and supporting the co-varying nature of LMA and %N regardless of CO_2:O_2 ratio. These findings suggest that atmospheric composition has a pronounced impact on LMA. Based on these results, we propose the hypothesis that atmospheric composition is an important taphonomic filter of the fossil leaf record. Further research is now required to test the significance of atmospheric composition versus other well-known taphonomic filters.

Key words: plant preservation; leaf taphonomy; atmospheric composition; LMA; fossil plants

Highlights

1 Mesozoic analogue taxa exhibit higher LMA with increasing CO_2:O_2 ratio

2 %N decreases with increasing LMA in different palaeo-atmospheric compositions

3 High CO_2 episodes in the geological past possibly increased fossil leaf preservation potential

1. INTRODUCTION

There are numerous factors, biological, physical and chemical, that influence whether or not a once living plant or animal enters the fossil record (Benton & Harper, 2009; Briggs, 2003; Butler et al., 2015; Kidwell, 2001; McNamara et al., 2012; Redelstorff & Orr, 2015). For plants, the majority of macrofossil assemblages are leaf litter (Greenwood, 1991), making leaves among the most common plant organs preserved in the fossil record. Therefore, a detailed understanding of how leaves are preserved and the taphonomic filters that act on leaf preservation in the fossil record is extremely important and has been the subject of much work over the last several decades. After climate and source vegetation, which both control the leaf litter available for preservation (Burham et al., 1992; Burham et al., 2005; Gastaldo and Staub, 1999; Greenwood, 1991; Spicer, 1989), depositional environment is most likely the primary control that determines preservation potential of leaf macrofossils (Ferguson, 2005; Gastaldo et al., 1987; Gastaldo & Demko, 2011; Gastaldo, 1989; Gastaldo et al., 1996; Gee et al., 2005; Greenwood, 1991; Spicer, 1989). Other factors that are known to impact on preservation potential include the chemical composition of plant organs (Briggs, 1999; Collinson et al., 1998; Retallack, 2011; Witkowski et al., 2012); premineralisation (Briggs, 1999; Channing & Edwards, 2003; Labe et al., 2012; Scott & Collinson, 2003) and the thickness of leaves (Gastaldo, 2001; Spicer, 1989). There are numerous other factors that can impact on the preservation potential of leaves in the plant fossil record and a summary of some of the key taphonomic filters is provided in Table 1. One factor that has not, to the best of our knowledge, been investigated for its effect on preservation potential of plant material in the fossil record is atmospheric composition at the time of leaf growth and deposition. Atmospheric composition has shifted dynamically throughout Earth history, and plants in turn have responded via morphological (Bacon et al., 2013; Beerling, 2005; Beerling et al., 2001; McElwain et al., 1999; Niklas, 1986; Haworth et al., 2011), ecophysiological (Boyce et al., 2009; Franks & Beering, 2009; Haworth et al., 2014; Haworth et al., 2015; Steinthorsdottir et al., 2012) and anatomical (Field et al., 2011; de Boer et al., 2012; Thomasson et al., 1986) adaptation. Here we ask, could functional adaptation to atmospheric composition in the past have influenced fossil leaf preservation potential?

Leaf mass per area (LMA) is an important functional trait of plants that expresses leaf dry-mass invested per unit of light-intercepting leaf area (Wright et al., 2004). LMA has been shown to increase when plants are grown in experimentally elevated concentrations of carbon dioxide (CO_2) (Ainsworth & Long, 2005; Poorter et al., 2009) and to decrease when grown in sub-ambient CO_2 (Temme et al., 2013). However, the possibility of changes in LMA, mediated by changes in atmospheric composition, as having a direct, if secondary, impact on preservation potential of fossil leaves, has not been considered. This is likely of significant importance because previous studies have identified leaf thickness as important to plant preservation (Gastaldo, 2001; Spicer, 1989) and therefore, if LMA increases or decreases depending on the atmospheric composition at time of growth, this may have a direct, secondary impact on preservation potential of fossil leaves. Atmospheric composition is known to have changed dramatically over the last 400 million years (e.g. Berner, 2006; Berner et al., 2007; Bergman et al., 2004) and within the Mesozoic concentrations of both CO_2 and O_2 are thought to have altered dramatically over both longer (e.g. Berner, 2006; Berner et al., 2007; Bergman et al., 2007; Belcher & McElwain, 2008) and shorter (e.g. Prochnow et al., 2005; Schaller et al., 2011; Steinthorsdottir et al., 2011; Weissert & Erba, 2004) timescales. Given that atmospheric composition is known to effect LMA and given that leaf thickness is known to be a factor that helps to determine plant preservation potential, this suggests that these changes in atmospheric composition may have led to shifts in LMA and thereby alteration of the preservation potential of leaves at different points in time. Most studies of LMA focus on crop plants or other angiosperms, which are not representative of Mesozoic or older fossil floras. Further, there are relatively few studies that focus on the effects of very high (>1,000ppm) CO_2 or changes in atmospheric oxygen (O_2) that characterize the Mesozoic Eon. This suggests that existing studies on LMA and atmospheric composition are likely not applicable to the Mesozoic as this would require considerable extrapolation, and highlights the need for experimental studies on plant responses to these atmospheric compositions. In particular, the effects of low O_2 on LMA have not been well-studied, and, as the Mesozoic is characterized by both high CO_2 and low O_2, the effects of low O_2 on LMA are essential to understanding how plants may have responded to these Mesozoic shifts in atmospheric composition.

In addition to responding to atmospheric composition, LMA is one of six co-varying functional traits that together make up the "worldwide leaf economic spectrum" (WLES) (Wright et al., 2004). The WLES traits provide a simple means of investigating ecosystem function; however, only LMA can be inferred in the fossil record (Royer et al., 2007; 2010; Haworth & Raschi, 2014; Blonder et al., 2014). Measuring LMA in the fossil record allows the investigation of how plants may have responded to major environmental upheavals (Blonder et al., 2014; Haworth & Raschi, 2014) and of plant evolution (Royer et al., 2010). However, whether or not the co-varying nature of the WLES traits is sustained in atmospheric compositions different to modern levels, has not been investigated. Among the key WLES traits are LMA and %N (percentage nitrogen) (and C:N (carbon:nitrogen) ratio). %N and C:N ratio provide information on how plants utilise available nutrients and link to overall plant function, as nitrogen is an essential component of many enzymes, including the photosynthetically essential enzyme Rubisco (Wright et al., 2004). If changes to atmospheric composition can significantly alter LMA and C:N ratios in a range of plants in a manner consistent with the predictions of the leaf economic spectrum (Wright et al., 2004), this would provide support for using LMA-based plant function reconstructions to investigate the ecology of palaeofloras.

The aims of this study were to: 1) investigate LMA and C:N responses of plants representative of an Early Mesozoic flora to different concentrations of atmospheric CO_2 and O_2; 2) determine whether altered atmospheric composition influences the paired responses between LMA and C:N , as predicted by WLES; and 3) consider how this may impact the preservation potential of leaves in the plant fossil record and interpretations of fossil floras.

2. MATERIALS AND METHODS

2.1 Simulated palaeoatmosphere treatments

Six nearest living equivalent (NLE) taxa were selected as analogues for abundant Early Mesozoic fossil taxa – *Agathis australis* and *Nageia nagi* were selected as NLEs for broad-leaved conifers, *Ginkgo biloba* was selected for ginkgophytes, *Lepidozamia hopei* and *L. peroffskyana* for Bennettitales and Cycadales, and *Dicksonia antarctica* for Mesozoic ferns. Examples of the leaves of each species are provided in Figure 1. Three plants of each species were grown in four Conviron BDW 40 walk-in controlled atmosphere and climate chambers under four different atmospheric treatments as follows: A control treatment was maintained at ambient concentrations of CO_2 (380ppm) and O_2 (20.9%) (CO_2:O_2 ratio of 0.0018); a low O_2 treatment with ambient CO_2 and sub-ambient O_2 at 13% (CO_2:O_2 ratio of 0.0029); a high CO_2 treatment was maintained at ambient O_2 and elevated CO_2 of 1,500ppm (CO_2:O_2 ratio of 0.0072); and a high CO_2/low O_2 treatment with CO_2 at 1,500 ppm and O_2 at 13% (CO_2:O_2 ratio of 0.0115). Collectively the four treatments provided a good range of hypothesized palaeoatmospheric conditions for the Early to Middle Mesozoic. The Early to Middle Mesozoic is characterized by high, but variable atmospheric CO_2 (Berner, 2006; Bergman et al., 2004; Royer, 2001; Royer et al., 2004; McElwain & Chaloner, 1995) and periods of potentially low O_2 (Belcher & McElwain, 2008; Belcher et al., 2010; Berner, 2006; Berner et al., 2007; Glasspool & Scott, 2010). Atmospheric treatment conditions within the chambers were monitored and controlled as described in Haworth et al., (2010).

2.2 Leaf Mass per area analysis

Leaf samples were taken from mature new growth material to ensure that the sampled leaves had grown and developed under the simulated palaeoatmospheric treatments. For *G. biloba*, *N. nagi*, and *A. australis,* approximately 20 leaves were randomly selected per plant from three plants per treatment and for the cycads and *D. antarctica*, one frond was selected per plant and 20 randomly selected pinnae from each frond were analysed for each of three plants per treatment. Each leaf or pinnae was photographed using a 10.1 megapixel Canon 1000D digital single-lens reflective camera that produced high-quality images with 3888 x 2592 pixel resolution. The leaves were then dried at 40°C in an oven until dry weight was achieved. The leaf and pinnae photographs were analysed using ImageJ (1.39u – documentation and downloads at website http://rsbweb.nih.gov/ij/, National Institutes of Health, Bethesda, Maryland, USA) to determine leaf area, and this was then used with the dry weight measurements to calculate LMA for each leaf and pinnae. Statistical analyses were performed in PAST (http://nhm2.uio.no/norlex/past/download.html).

2.3 Carbon:Nitrogen (C:N) analysis

A sub-set of three leaves or pinnae from each plant in each treatment underwent C:N analysis to determine the effect of atmospheric composition on nutrient uptake and carbon storage. Each sample was ground to a fine dust in a ball mill, with all implements and the ball mill canisters cleaned thoroughly using acetone and a sonic water bath between samples. For *D. antarctica*, the individual pinnae were too light to be analysed separately, so the entire selected frond was used. Powdered samples were analysed for % carbon, % nitrogen and C:N ratio in an Elementar Vario Micro Cube. Statistical analyses were performed in PAST (http://nhm2.uio.no/norlex/past/download.html).

2.4 Meta-analysis of angiosperm LMA values under different atmospheric composition

In order to determine if the patterns of LMA response to atmospheric composition were specific to the gymnosperms in this study or more commonly identified in angiosperms, we extracted LMA values for C3 angiosperms from the recent meta-analysis of Temme et al., (2013). This study was chosen as it compiled a large number of previous studies and recorded both LMA and CO_2 data in easily extracted formats. Only studies reported within Temme et al., (2013) that clearly reported LMA or SLA (which was then converted to LMA) for both a control and at least one treatment for at least two C3 species were included in this analysis. An average LMA was calculated for all species across studies that had been grown in the same atmospheric composition and the percentage deviations from the control and CO_2:O_2 ratios were calculated for each study (list of studies in Supplementary material 1). None of the studies reported varying atmospheric O_2, so an ambient level of 20.9% was assumed for all studies. This is a small-scale analysis not meant to be exhaustive that aimed to determine if a small group of angiosperms showed a similar response to that observed for the gymnosperms in the current study and was kept purposely coarse with no distinction made between species.

3. RESULTS

3.1 LMA responses to atmospheric composition

3.1.1 Control treatment

All plants produced new growth and grew well in the control treatment with no signs of stress (see Table S1 in Supplementary material 2 for chlorophyll fluorescence (Fv/Fm) data). Figure 1 and Table 2 show the range of LMA for each species (raw data for all LMA values in all treatments presented in Supplementary material 1). The six species can be placed into four functional groups as described by Poorter et al., (2009) – evergreen gymnosperms (*A. australis* and *N. nagi*), deciduous trees (*G. biloba*), evergreen shrubs (*L. hopei* and *L. peroffskyana*) and ferns (*D. antarctica*). In Figure 2, panel (i) in each box shows the LMA range for the relevant functional group (redrawn from Poorter et al., 2009) for each species as a comparison to the values obtained in each treatment in panel (ii). As shown in Figure 2 and Table 2, the mean LMA values of each species in the control treatment (161 g m^{-2} for *N. nagi*; 224 g m^{-2} for *A. australis*; 84 g m^{-2} for *G. biloba*; 128 g m^{-2} for *L. hopei*; 138 g m^{-2} for *L. peroffskyana*; 82 g m^{-2} for *D. antarctica*) were observed to be within the 25–75 percentiles recorded by Poorter et al., (2009) for each functional group.

Although chamber experiments are known to underestimate LMA in many species, compared to the same species growing in a natural environment (Garnier & Freijsen, 1994), each of the species in this study had LMA values similar to those expected based on their functional group growing in the wild.

3.1.2 Simulated palaeoatmospheric treatments

Leaf mass per area responses of the five gymnosperm and one fern species to different palaeoatmospheric treatments are shown in Figure 2 and Table 2 (Supplementary material 2 shows detailed Kruskal Wallis analysis for all species in each treatment; theKruskal Wallis analysis was used here because some data were non-normally distributed.). The most statistically significant and consistent LMA response was observed in the high CO_2/low O_2 treatment (highest CO_2:O_2 ratio), where all species showed a large increase in LMA values significant at $p < 0.001$. The LMA response in the high CO_2 treatment also showed a strong tendency to increase across the species examined with the exception of the evergreen gymnosperms (Fig 2), which have the highest LMA values in the control treatment. All species that showed an increased LMA under high CO_2 did so significantly ($p < 0.001$). Species in the low O_2 treatment showed the least consistent LMA response: three species increased LMA – *A. australis* at $p < 0.05$ and *L. peroffskyana* and *D. Antarctica* at $p < 0.001$; *L. hopei* decreased LMA at $p < 0.05$, and *N. nagi* and *G. biloba* showed no significant response to LMA in this treatment. The lack of consistency within functional groups and between the two most closely related species (the two cycads) suggests that plant LMA responses to low O_2 are not highly conserved between these taxa.

3.2 C:N responses

3.2.1 Control treatment

In the control treatment, C:N ratio analysis revealed a wide range of C:N values between the different species, as expected based on functional type (raw data for all C:N, %N, and %C values in all treatments presented in Supplementary material 1 and Supplementary material 2 shows detailed Kruskal Wallis analysis for all species in each treatment). *Agathis australis* and *N. nagi*, the two evergreen gymnosperms with high LMA values, had C:N mean values of 74.45 and 80.6, respectively, while the two evergreen shrubs, *L. hopei* and *L. peroffskyana*, both with lower mean C:N values of 35.91 and 29.71, respectively, potentially due to the presence of nitrogen fixing bacteria in their roots (Halliday & Pate, 1978). *G. biloba*, the deciduous tree, had a mean C:N value of 73.35 and *D. antarctica*, the fern, had a mean C:N value of 37.92. This range of C:N values in the control treatment was as expected.

3.2.2 Simulated palaeoatmospheric treatments

C:N ratios increased significantly ($p < 0.05$) in all species when grown in the high CO_2/low O_2 treatment (Figure 3, Table 3; Supplementary material 1 & 2) with the exception of *D. antarctica*. The lack of significant increase in *D antarctica* was likely due to a smaller number of samples available for this species (3 samples per plant versus 9 per plant for the other species; see methods). The pattern of responses in the high CO_2 treatment was less consistent, with four of six species showing an increase in C:N ratio, three (*A. australis*, *G. biloba*, *L. peroffskyana*) significantly at $p < 0.05$ (Figure 3, Table 2) and *D. antarctica* non-significantly. The foliar C:N ratio of *N. nagi* declined, which was contrary to what was expected, but was likely due to the surprising rise in %N in this treatment for this species (Table 4, Appendix 2). *Lepidozamia hopei* showed no significant change in C:N ratio compared to the control in the high CO_2 treatment. Apart from *A. australis*, which showed an increase in C:N at $p < 0.05$, there were no significant responses compared to the control in the low O_2 treatment.

3.3 Comparison of LMA and C:N responses

3.3.1 Control treatment

According to the WLES paradigm (Wright et al., 2004), leaves with higher LMA are expected to have lower nitrogen content, which equates to higher C:N ratios. Figures 2 and 3 and Tables 2-5 show that in the control treatment, this prediction was generally met. The species with high LMA (above 150 g m^{-2}), such as *A. australis* and *N. nagi*, had lower mean %N and higher C:N ratios than the species with low (below 100 g m^{-2}), such as *L. hopei* and *L. peroffskyana*, or very low, such as *D. antarctica*, LMA values. The exception to this is *G. biloba*, which had a low mean LMA of ~84 g m^{-2} but also a low %N (0.68%) and a high C:N ratio (mean value ~73) compared to the other species in the study.

3.3.2 Simulated palaeoatmospheric treatments

Within the simulated palaeoatmospheric treatments, where significant LMA changes were observed, significant %N and C:N ratio changes were also usually observed (Figure 2 & 3, Tables 2–5), although this is not always the case. *Dicksonia antarctica* showed changes to %N and C:N ratio that were consistent with WLES predictions, but these variations were not statistically significant, likely due to the small sample size for C:N analysis. In some cases, a statistically significant change in LMA was not matched by a significant change in %N or C:N ratio. For example, the significant ($p < 0.05$) decrease in LMA for *L. hopei* in the low O_2 treatment was not matched by a significant rise in %N and the significant increase ($p < 0.001$) in LMA for the same species in high CO_2 was not matched by a decrease in %N. The high CO_2/low O_2 treatment showed the most consistent and statistically significant suite of responses in terms of WLES, with all species (except for *D. antarctica*) increasing LMA, decreasing %N and increasing C:N ratios at $p < 0.05$ or less.

3.4 Generalized response of leaf economic traits to increasing CO_2:O_2 ratio

3.4.1 Generalized LMA responses to atmospheric composition

The effect of atmospheric composition on each individual species is shown in Figure 2 and 3, but in order to determine how atmospheric composition effected all species within one treatment, each treatment was considered as a “mini-ecosystem” and an average value for LMA, C:N and %N for all species within each treatment was calculated. Figure 4 shows the mean values for each trait with a standard least squares regression for both raw data (Figure 4A) and % deviation from the control (based on mean values) (Figure 4B) against atmospheric composition, expressed as a CO_2:O_2 ratio. Figure 4A shows that there was a general trend of increasing LMA and C:N ratio ($p < 0.05$; $R^2 = 0.96$) and a decreasing, but not significant ($p = 0.061$; $R^2 = 0.88$), trend in %N with increasing CO_2:O_2 ratio. Figure 4B shows a similar pattern, with an increasing deviation from the control treatment as CO_2:O_2 ratio was increased between treatments, suggesting that the effect of increasing LMA and C:N ratio and decreasing %N becomes more apparent with a greater CO_2:O_2 ratio. The high R^2 values of each regression are likely a result of small sample size, rather than a generalized strong response to increasing

CO_2:O_2 ratio, and the lack of significance for %N is also likely a function of sample size. Additional treatments would be needed to make this analysis more robust. However, the findings show that, for this group of plants, as CO_2:O_2 ratio increases, LMA and C:N ratio can be expected to increase, while %N decreases.

3.4.2 Generalized angiosperms LMA responses to atmospheric composition

In order to determine if the observed increase in LMA with increasing CO_2:O_2 ratio shown in Figure 4A and 4B could be identified in other taxa, we extracted LMA values for C3 angiosperms and calculated "mini-ecosystem" mean responses from the meta-analysis of Temme et al., (2013) to extend the data set. Figure 4C shows the results for this increased range of species and experimental treatments to variation in CO_2:O_2 ratio. Once again a general increase was observed in the % change of LMA reported relative to a control with increasing CO_2:O_2 ratio (dark grey line; $p < 0.001$; $R^2 = 0.56$). When the species from the current study were included in the regression (pale grey line Figure 4C; data from both this study and Temme et al., 2013), the relationship was slightly improved (pale grey line; $p < 0.001$; $R^2 = 0.63$). Although this is not a very strong a relationship, the statistical significance of the regression when such a diverse array of species and experiments are considered together suggests that the response of increasing LMA with increasing CO_2:O_2 observed in the current study is likely to be observed in a wide range of other species. However, the angiosperm data are all from much lower CO_2 concentrations and none varied O_2, so although this analysis extends the range of species and treatments slightly, more high CO_2 and low O_2 studies on a diverse range of species are needed to fully test this hypothesis. Additionally, although this increase in LMA with increasing CO_2:O_2

ratio is a physical response and unlikely to be much affected by phylogenic affinity, there remains the possibility of a phylogenetic effect on these data. A full phylogenetic analysis of these data was far beyond the scope of this study, but future work will need to address this before the relationship can be fully accepted as legitimate. Regardless, Figure 4C highlights that once a CO_2:O_2 ratio of above 0.003 is reached, most species, irrespective of phylogenetic affinity, increase LMA by at least 15–20%.

4. DISCUSSION

4.1 LMA responses to changing atmospheric composition

Leaf mass per area was found to increase significantly with increasing CO_2:O_2 ratio for a range of species that act as nearest living equivalents for abundant Mesozoic taxa. This has significant implications for understanding both how atmospheric composition may have interacted with ancient floras and how significant and large increases in LMA may impact on leaf fossil preservation potential (see 4.3).

The highly significant increase in LMA in the high CO_2/low O_2 palaeoatmospheric treatment highlighted that atmospheric composition has a conserved effect across an evolutionary diverse group of plants. LMA values in experimental laboratory conditions are generally considered to be slightly lower than values obtained for the same species grown in natural conditions (Poorter et al., 2009), likely due to decreased daily photon irradiance and more variable and lower temperatures (Garnier & Freijsen, 1994). The simulated palaeoatmospheric treatments in controlled environments aimed to create a closer match to natural conditions, particularly light and temperature variations, than standard chamber or glasshouse experiments, and so should at least have reduced this effect.

Comparison of the LMA values obtained in this study to LMA values for plants grown under natural conditions was difficult because most species investigated do not appear to have been previously reported in the literature. No reported LMA values could be found for *A. australis*, *N. nagi*, *L. peroffskyana*, *L. hopei* or *D. antarctica*. This study therefore, provides the first estimate of LMA under control conditions for these species (Figure 2, Table 2), as well as the first reported LMA values for these species under different atmospheric compositions. For *G. biloba* reported LMA values under modern atmospheric conditions range from ~51.64 g m^{-2} (He et al., 2010), to ~84-94 g m^{-2} (Leigh et al., 2010), to 53.7 to 155.9 g m^{-2} (Haworth and Raschi, 2014), to ~91.5–136g m^{-2} (Sack et al., 2006). The values from the control treatment (mean ~84 gm^2) are similar to these previously reported LMA values, although they are, as expected for chamber experiments, towards the lower end of the reported range of values. The similarity between LMA values for *G. biloba* from this study to previously published LMA values, support indications that the simulated atmospheres in the controlled environments produced realistic LMA values and suggests that the values for the other species and other treatments are at least broadly in line with, if slightly below, the values that would be obtained under natural conditions. This is particularly interesting in the context of results from the high CO_2/low O_2 treatment where LMA values increase greatly (~30% or more) for all species in the study. This suggests that during periods of elevated CO_2 (> 1,000 ppm), plants likely had considerably higher LMA values than at present and possibly greater values than those reported here under CO_2 conditions of 1,500 ppm.

This study adds to the current understanding of how plants, particularly non-angiosperms, respond to different atmospheric compositions. In particular, responses to elevated (e.g. >1,000ppm) CO_2 and low (<20%) O_2 are rare or absent for these, and indeed most, species in the literature. The findings of increased LMA in enriched CO_2 environments are in line with many previous angiosperm-based studies (e.g. Aguera et al., 2006; Cao et al., 2008; Cunniff et al., 2008; Curtis et al., 2000; Donnelly et al., 2001; Gilbeaut et al., 2001; Harmens et al., 2000; Roumet et al., 2000; Sigurdsson et al., 2001; Tricker et al., 2004; Volin et al., 2002; Vuorinen et al. 2004). Figure 4 suggests that with increasing $CO_2:O_2$ ratio, plants, regardless of their functional group, respond by increasing LMA. This is observed with a rise in CO_2 alone but is further magnified in this study when high CO_2 is combined with low O_2. It is likely that a similarly magnified response would be observed in angiosperms, but this was beyond the scope of the current study and no study that exposed angiosperms to combined high CO_2 and low O_2 could be found in the literature.

The low O_2 treatment elicited a wide-range of species-specific responses. Research into plant responses to below ambient atmospheric O_2 is fairly limited. Migge et al., (1999) identified a decrease in overall plant size, a reduction in leaf expansion, a reduction in leaf area, and an increase in LMA for *Nicotiana tobacum* plants exposed to low O_2, and Musgrave & Strain, (1988) identified an increase in dry matter of *Triticum aestivum* plants exposed to low O_2 and an even greater increase in dry matter when low O_2 was combined with elevated CO_2. However, both of these studies exposed plants to O_2 levels below 5%, which is far lower than at any time in Earth history when embroyphytes have existed, making the results difficult to apply in terms of plant evolution. In the current study, the lack of consistency within and between functional groups and between the two most closely related species (*L. hopei* and *L. peroffskyana*) in the low O_2 treatment (but not the other treatments) suggests that plant responses to low O_2 are not very highly conserved. This may be due to a lack of exposure to very low O_2 atmospheres alone (rather than in conjunction with high CO_2) in the evolutionary history of these plant groups (Berner, 2006; Haworth et al., 2011; Shinde et al., 2015) or it may be due to different plants responding to low O_2 through different mechanisms (Shinde et al., 2015). How plants sense and respond to decreased O_2 levels is a topic of current research and not well-understood outside of the angiosperms. Recently, group VII ethylene response factors (ERFVIIs) have been identified as direct oxygen sensors in angiosperms including *Arabidopsis* (Nakano et al., 2006; Licausi et al., 2013; Gibbs et al., 2015) and *Oryza* (Nakano et al., 2006; Gibbs et al., 2015). Whether ERFVIIs are highly conserved or have a similar role in other plant groups is uncertain, as although the same group of ERFs have been identified as oxygen sensors in both *Arabidopsis* and *Oryza*, and ethylene has been identified as having a role in oxygen sensing in the moss *Physcomitrella patens* (Yasumura et al., 2012), a different response has been identified in microalgae (Banti et al., 2013). The mechanism involved in oxygen sensing for gymnosperms has yet to be identified. This could suggest that variation in the ability to sense lower levels of O_2 may have a role in the species-specific responses in terms of LMA variation in the low O_2 treatment observed in this study.

Overall, the diverse group of plants in this study revealed remarkably consistent responses to increasing CO_2:O_2 ratio in terms of LMA, suggesting that most plants will have a similar response, particularly to very high CO_2 and high CO_2 with low O_2, atmospheric combinations that were not uncommon during the last 400 years of Earth history. The magnitude of LMA increase between the control treatment and the high CO_2/low O_2 treatment is particularly significant. Figure 5 compares the shift in LMA values for one high LMA species (*A. australis*) and one lower LMA species (*G. biloba*) between the control treatment and the high CO_2/low O_2 simulated palaeoatmospheric treatment to the range of LMA values observed in a variety of functional groups in ambient conditions (redrawn from Poorter et al., 2009). This demonstrated that the difference in LMA observed between the control and high CO_2/low O_2 treatments is equivalent to taxa shifting at least one functional group higher in terms of LMA values, and in the case of *A. australis* two groups higher. Such a large-scale shift in LMA could have major implications for interpretation of palaeoecology and palaeoecosystem function, with an increase in LMA leading to decrease nutrient availability (Norby et al., 1999; Wright et al., 2004), a reduction in the palatability of leaves for herbivores (Cotrufo et al., 1998; Currano et al., 2008; 2009; Royer et al., 2007), and a slowing of biogeochemical cycling through a slowing of leaf decomposition (Cotrufo et al., 1998; Cornelissen et al., 1999; Cornwell et al., 2008) as leaves would take longer to return nutrients and carbon to soil.

4.2 WLES responses to atmospheric composition change

There are few previous data sets available in the literature to determine a base-line for C:N or even %N under controlled or field conditions for the species examined here. For *A. australis* growing in New Zealand forests, Enright (2001) recorded a value of ~1% nitrogen and Sylvester (2000) recorded values of 0.43% N and a C:N ratio of 62. In the current study, mean values of 0.69% N and a C:N ratio of 74 were recorded for *A. australis*. No other reports of C:N ratio or %N values for the species in this study could be identified in the literature either for ambient conditions or under enriched CO_2 conditions, making the values reported here the first time these responses have been considered in the literature.

The responses both within species (Figures 2 and 3) and between species (Figure 4) in this study generally conformed with the overall predictions of WLES, that higher LMA should lead to increasing C:N and decreasing %N and lower LMA should lead to decreasing C:N and increasing %N. The most significant responses were identified in high CO_2 and in high CO_2/low O_2 where increasing LMA usually resulted in increasing C:N and decreasing %N. This is in line with previous studies in a range of species (e.g. Aguera et al., 2006; Harmens et al., 2000; Cao et al., 2008).

The few exceptions, for example increased %N and increased LMA compared to the control treatment for *L. peroffskyana* growing in the low O_2 treatment and decreased %N and no change in LMA compared to the control for *A. australis* in the high CO_2 treatment appear to be highly species specific outliers. Donovan et al., (2011) noted that selective pressures were likely to have played a larger role in the evolution of WLES than genetic constraints. The observed responses of LMA and %N generally conform to the WLES predictions but, as in the two examples above, this is not always the case. Donovan et al., (2011) note that while genetics do not preclude trait pairings contrary to the pattern observed within WLES, environmental pressures likely do. This is relevant when considering the increase in both LMA and %N for *L. peroffskyana* in the low O_2 treatment, compared to the control. It is unlikely that this result would be observed in a natural environment, as high %N in thick leaves is expensive in terms of carbon usage. It would also likely increase herbivory of these carbon-expensive leaves. However, controlled environment experiments protect plants from herbivory and also reduce competition, potentially permitting the development of unlikely trait pairings (Donovan et al., 2011). However, for most trait pairs in this experiment, the responses of plants were generally in line with WLES predictions.

Some species also showed a large increase in the range of values for LMA in some treatments. This is particularly noticeable in the LMA values for *L. peroffskyana.* Ward & Kelly (2004) previously suggested that CO_2 can act as a selective agent for plant populations and Ward et al., (2002) identified a link between specific genes and the alteration of flowering time in *Arabidopsis thaliana* in elevated CO_2. As selection acts on variation, the increase in trait variation observed as CO_2:O_2 ratio increased in some species suggests that changes in atmospheric composition over geological time may have acted as a selective pressure for WLES traits.

4.3 LMA, atmospheric composition and interpreting the fossil record

Previous studies have identified a clear relationship between increasing LMA under elevated atmospheric CO_2 (e.g. see reviews in Ainsworth & Long, 2005; Poorter et al., 2009) and decreasing LMA under sub-ambient atmospheric CO_2 (e.g. Temme et al., 2013). However, the possible “knock-on” implications of this in relation to how increasing or decreasing LMA may impact preservation potential of leaves, through changes in average leaf density and/or thickness, in the fossil record have not been considered. Increased leaf thickness is known to increase preservation potential (Gastaldo, 2001; Spicer, 1989) and, as this study suggests that times of higher CO_2:O_2 ratio select for high LMA leaves, this could result in a greater proportion of high-quality plant cuticle and compressed mesophyll tissue preserved either as near complete compression fossils or as good-quality cuticle fragments at times of higher CO_2:O_2 ratio in Earth history (Figure 6).

This of course, would be a secondary control on taphonomy, with depositional environment and rapidity of burial remaining the most defining factors for preservation (Ferguson, 2005; Gastaldo et al., 1987; Gastaldo & Demko, 2011; Gastaldo et al., 1989; Gastaldo et al., 1996; Gee et al., 2005; Greenwood, 1991; Spicer, 1989) (Table 1). However, if atmospheric composition does have a role in plant preservation potential, then, within suitable depositional environments, such as crevasse splay deposits (Gastaldo et al., 1996), certain time periods may be more or less likely to have better or worse, or more or fewer, plant leaves preserved in depositional environments than others.

The findings of this study suggest that, in particular, CO_2:O_2 ratios of approximately 0.003 or greater may increase LMA by between 10 and 50% (Figure 3). Figure 6 highlights times of likely "high LMA world" and "low LMA world" over the last 450 million years calculated against Berner (2001) GEOCARB III values for CO_2 and O_2. A "high LMA world" is defined as having a CO_2:O_2 ratio of 0.003 or greater and a "low LMA world" is defined as having a CO_2:O_2 ratio below this.

Although this may be useful for investigating large-scale variation in preservation, the greatest utility may be in investigating finer time scales. Retallack (2011) has previously suggested that exceptional preservation is correlated to super elevated atmospheric CO_2. Therefore at times of predicted low O_2 (below 20%) and during episodes of sudden increases in CO_2, the increase in LMA would be most noticeable in terms of preservation, particularly across ecologically disrupted boundaries, such as major extinctions or other periods of significant ecological change. The corollary is also true, and at times of high and falling or low CO_2, leaf fossil preservation may be expected to be poorer even in depositional environments that favour plant fossil preservation by comparison to the same environment under higher CO_2 conditions. In particular, times with sudden changes in atmospheric composition recorded over isotaphonomic beds would be expected to show a change to the quality of preservation as atmospheric composition changes. This could either help to increase or decrease confidence of palaeoecological interpretations. For example, a palaeoecological analysis of leaf macrofossils during a period of rising CO_2 across isotaphomomic beds that shows a decrease in morphospecies but a rise in preservation quality would help to support claims of declining biodiversity, whereas a rise in diversity could be in part due to an increase in preservation potential of species growing in a high and rising LMA world. Regardless, consideration of changes to atmospheric composition alongside other taphonomic filters may help to increase confidence in biodiversity analysis across key boundaries and improve understanding of ecosystem responses to environmental change in Earth history.

Overall, the findings of this study highlight some interesting hypotheses for further investigation – if simulated Mesozoic atmospheric compositions can so significantly alter LMA and C:N ratios of a diverse group of NLE taxa, can this change lead to an impact on preservation potential of plant leaves in the fossil record? In addition, should atmospheric composition be considered as a second order taphonomic filter for fossil leaves and should atmospheric composition variation, in terms of the CO_2:O_2 ratio, be considered alongside other taphonomic filters, such as depositional environment, chemical alteration and chemical composition in order to generate a more complete understanding of the plant fossil record? These are all testable research questions that can, hopefully, be addressed through further studies of fossil collections by either comparing preservation quality to targeted reconstruction of LMA (Royer et al., 2007; 2010; Blonder et al., 2014; Haworth & Raschi, 2014) or comparison of preservation quality in similar depositional environments at times of different CO_2:O_2 ratios.

5. CONCLUSIONS

This study reports the first LMA and C:N values in simulated palaeoatmospheric controlled environment experiments for a range of plants considered as nearest living equivalents of early

Mesozoic floras, increasing understanding of how non-angiosperm species respond to elevated CO_2.

The results of the simulated palaeoatmospheric treatments reveal a consistent response within this diverse group of NLE species to changing atmospheric composition. The consistent increase in LMA and C:N ratio and decrease in %N across all six species in this study suggests that this is likely to be a highly conserved response, common across a wide range of plant taxa. This interpretation is further supported by the responses of angiosperms to a variety of atmospheric conditions that also show an increase in LMA with increasing CO_2:O_2 ratio. These experimental findings suggest that plants may significantly alter their LMA over time in response to changing atmospheric composition, which raises the possibility that atmospheric composition induced changes to LMA may lead to increased preservation potential of leaves at times of high CO_2:O_2 ratio in the geological record. If future work determines this to be the case, then consideration of the impact of atmospheric composition on leaf preservation potential in the fossil record may help constrain uncertainty associated with patterns of fossil plant diversity and macroecological change over the last 400 million years.

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FIGURE LEGENDS

Figure 1:
Example leaves for each species in the experimental study. A) *Agathis australis*; B) *Nageia nagi* ; C) *Ginkgo biloba*; D) *Dicksonia antarctica;* E) *Lepidozamia peroffskyana;* F) *Lepidozamia hopei* . Scale bars are all 1 cm. All leaves are from the control treatment.

Figure 2:
Box plots showing the range of LMA values for each species in each palaeoatmospheric treatment. Panel (i) shows the range of LMA values for the functional group to which each species belongs (redrawn from Poorter et al., 2009) and panel (ii) shows the range of LMA values for each species in each palaeoatmospheric treatment. Box represents the upper 25 percentile, median value and lower 25 percentile and whiskers show the range of the data. Black dots show outliers. Light grey boxes indicate a statistically significant difference from the control of $0.05 > p > 0.001$. Dark grey boxes indicate a statistically significant difference from the control of $p < 0.001$.

Figure 3:
Box plots showing the range of C:N ratio values for each species in each palaeoatmospheric treatment. Box represents the upper 25 percentile, median value and lower 25 percentile and whiskers show the range of the data. Black dots show outliers. Light grey boxes indicate a statistically significant difference from the control of $0.05 > p > 0.001$. Dark grey boxes indicate a statistically significant difference from the control of $p < 0.001$.

Figure 4:
Average responses of all species within each treatment to increasing CO_2:O_2 ratios A) raw data; B) Mean % deviation from the control treatment; C) regression including C3 angiosperm data (from Teeme et al., 2013) dark line is only C3 angiosperm data (Temme et al., 2013) and pale line includes both the angiosperm data and data from the current study. Stars indicate that regression is significant at $p < 0.05$.

Figure 5:
Comparison of placement of *Agathis australis* and *Ginkgo biloba* LMA values in control treatment and high CO_2/low O_2 palaeoatmospheric treatment in relation to functional group LMA values (redrawn from Poorter et al., 2009)

Figure 6:
Possible timing of “high CO_2 world” (dark grey) with high preservation potential and “low CO_2 world” (light grey) with lower preservation potential for plant fossil leaves based on a CO_2:O_2 cutoff ratio of 0.003 and atmospheric composition based on Berner (2006)

SUPPORTING INFORMATION HEADINGS

Supporting material 1: Raw data for LMA, C:N, %N, %C and for angiosperm comparison in Figure 4 (data from Temme et al., 2013)

Supporting material 2: Detailed Kruskal-Wallis and Mann Whitney U pair-wise comparisons for each measured trait in each species across all simulated palaeoatmospheric treatments.