Effects of multiple environmental stressors on litter chemical composition and decomposition

Thesis submitted for the degree of Doctor of Philosophy

by

Matthew William Dray BSc MSc

School of Biosciences

Cardiff University

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"Twigs and reason are the universal law, good for all men…
twigs and talk will teach you to live and think better."

Richard Llewellyn
How Green Was My Valley (1939)

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Abstract

Tree litter is a key basal resource in temperate deciduous woodlands and streams that drain them. Litter decomposition promotes carbon and nutrient cycling, fueling woodland food webs. Research to date has not thoroughly explored how ongoing environmental changes affect this process. This study used microcosm and field experiments to investigate how multiple stressors (urban pollution, elevated atmospheric CO_2 and stream acidification) affected litter chemical composition, invertebrate consumption, and terrestrial and aquatic mass loss. Leaf litter chemical composition differed between ambient- and elevated-CO_2 litters, and between rural and urban litters, but the direction of these responses was complex and differed between experiments. In microcosms, leaf litter consumption by terrestrial and aquatic invertebrate detritivores was species-specific. After exposure to a woodland floor or headwater streams, urban litter broke down faster than rural litter, while CO_2 treatment did little to influence mass loss. The abundance, richness and diversity of terrestrial and aquatic invertebrates associated with leaf litter generally declined from 28 to 112 days in the field. Taxon richness and diversity were generally higher in elevated- than ambient-CO_2 leaf litter through time, while urban leaf litter had greater diversity than rural litter after 112 days only. Abundance was greater in the circumneutral than the acid stream. Aside from leaf litter, small woody debris was also affected by CO_2 treatment: elevated-CO_2 twigs had a greater concentration of nitrogen and lignin, and broke down faster than ambient-CO_2 twigs on a woodland floor and in headwater streams. This work highlights the complexity of invertebrate- and ecosystem-scale responses to the effects of multiple environmental stressors, with implications for nutrient cycling and food webs. Urban pollution may have a greater influence on litter chemical composition than CO_2 treatment, while effects of growth condition may be more important than stream acidity in influencing decay and invertebrate communities.

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List of abbreviations

AFDM	Ash-Free Dry Mass
ANOVA	Analysis of Variance
C/N	Carbon/Nitrogen ratio
DCW	Dry Cell Wall
DM	Dry Mass
GLM	General Linear Model
GLMM	General Linear Mixed Model
LSM	Least Square Mean
NMDS	Non-metric Multidimensional Scaling
NPP	Net Primary Productivity
PAM	Pulse Amplitude Modulated
PERMANOVA	Permutational Analysis of Variance
SEM	Standard Error of the Mean
SIMPER	Similarity Percentages
SWD	Small Woody Debris

1. General introduction

1.1 Litter in temperate forests

Temperate forests cover over 1.4×10^9 hectares of the Earth's surface (Reich & Bolstad 2001) from 25–55° N and counterpart regions of the Southern Hemisphere. They are composed of an estimated 312.5 Mg ha^{-1} of aboveground biomass (Lefsky *et al.* 2002), dominated typically by trees of the genera *Acer*, *Betula*, *Fagus*, *Populus* and *Quercus*. The total Net Primary Production (NPP) of this habitat type is an estimated 2.2×10^{15} g $year^{-1}$ of carbon (Melillo *et al.* 1993), with mean carbon storage estimated at 0.72 Pg $year^{-1}$ (Pan *et al.* 2011). This makes temperate forests an important carbon sink (Luyssaert *et al.* 2007, 2008). Foliage and woody structures are particularly important carbon storage tissues (Mooney 1972; Aber & Melillo 2001; Lamlom & Savidge 2003; Lorenz & Lal 2010) given their relatively high concentrations of structural compounds such as celluloses and lignin (Taiz & Zeiger 2006; Chave *et al.* 2009; Novaes *et al.* 2009), and non-structural carbohydrates, such as starch, sucrose and glucose (Hoch, Richter & Körner 2003). These tissues also store important nutrients, such as nitrogen, phosphorus and other mineral ions, including potassium, calcium and magnesium (Chave *et al.* 2009).

The majority of deciduous forest NPP escapes herbivory (Cyr & Pace 1993) and enters the detrital pathway as litter (Hairston Jr & Hairston Sr 1993; Cebrian 1999; Thomas & Packham 2007). In one aspen forest in southwestern Alberta, Canada, this amounted to a leaf litter standing stock of 250 g m^{-2}, encompassing 3.7% of the total organic matter in the ecosystem (Louisier & Parkinson 1976). In terms of input rates, Gosz *et al*. (1972) calculated that 5,702 kg ha^{-1} of litter per year entered the Hubbard Brook Experimental Forest, New Hampshire, USA, and that it was comprised of 50% leaf material and 25% small woody material. Such litter inputs can vary spatially and temporally, as leaf litter tends to fall seasonally in autumn (Gosz, Likens & Bormann 1972; Abelho & Graça 1998; Abelho 2001), while woody debris inputs are more sporadic, depending on local tree death and extreme weather events (Kirby *et al.* 1998; Berg & McClaugherty 2008).

As well as terrestrial input, litter is an important source of energy for headwater streams adjacent to temperate deciduous woodlands. Organic material can fall into water directly (vertically) or via the forest floor (laterally), with the former providing the major contribution (Benfield 1997; Pozo *et al.* 1997). Fisher and Likens (1973) found that 99% of the annual energy input to a headwater stream – approximately 6,039 Kcal m^{-2} $year^{-1}$ – was allochthonous (derived externally). The quantity of litter entering streams can be highly variable, however, with a review by Abelho (2001) finding that input rates ranged between 3 and 761 g m^{-2} $year^{-1}$ in mixed deciduous forests. Abelho and Graça (1998) showed that these inputs are comprised largely of leaves (62%), followed by twigs (16%). Given this difference, studies of organic matter decomposition have tended to focus on leaves rather than woody debris (Harmon *et al.* 1986; Webster *et al.* 1999; Abelho 2001). Only a few studies have indicated that woody debris can make a large contribution to total litterfall. For example, leaves and branches contributed approximately equally to the 4,730 Kcal m^{-2} of energy falling as organic detritus at Bear Brook, USA (Fisher & Likens 1973).

Of the studies that consider woody litter, Small Woody Debris (SWD; e.g. twigs) is under-studied compared to Large Woody Debris (LWD; e.g. logs and branches). Small woody debris is important to standing stocks of detrital material, particularly in high-order streams (Bilby & Ward 1989): one temperate deciduous forest contained an estimated 5.06 Mg ha^{-1} of fragments less than 3 cm in diameter (Onega & Eickmeier 1991), while 20% of litter entering a temperate forest stream was comprised of twigs and branches, resulting in a contribution of 62% to the standing stock of coarse benthic organic matter (Abelho & Graça 1998). Retention of SWD is also higher than for leaf litter, making it a locally stable resource in the locations that it falls (Trotter 1990; Wallace, Whiles & Eggert 1995). It is, however, generally patchy in time and space, making it difficult to measure and extrapolate its role in carbon storage at the forest scale. It is also difficult to reach consensus when variable definitions have been used, encompassing material with diameters of <10 cm (Harmon *et al.* 1986), <5 cm (Kirby *et al.* 1998), <2.5 cm (Thomas & Packham 2007) and 0.4–0.7 mm (Dearden *et al.* 2006). Proxies, such as tongue depressors (Arroita *et al.* 2012), veneers (Hofer & Richardson 2007), wood chips (Melillo *et al.* 1983) and

ice-cream sticks (Sinsabaugh *et al.* 1992) are also used in place of field-collected or greenhouse-grown SWD, all of which may have different dynamics to natural SWD.

As well as its energetic importance, litter affects the physical habitat and nutrient storage of temperate forests and adjacent streams. A meta-analysis by Xu *et al*. (2013) showed that removing the litter layer of the forest floor can increase soil temperatures and reduce soil moisture, while decreasing the Carbon-Nitrogen ratio (C/N) and total carbon and nitrogen content. These outcomes can be partially reversed by litter addition, but both soil moisture content and the total nitrogen content of the litter layer did not change in such studies. Coverage of the woodland floor by litter also influences competition between trees by reducing germination, establishment, species richness and aboveground biomass (Xiong & Nilsson 1999).

In freshwaters, woody litter plays an important role in channel stability (Bilby 1984) and morphology (Harmon *et al.* 1986), affecting stream hydraulics and habitat formation (Abbe & Montgomery 1996; Beechie & Sibley 1997). Much of the litter in headwater streams is incorporated into woody debris dams (Bilby & Likens 1980; Smock, Metzler & Gladden 1989; Flores *et al.* 2011), which may contain as much as 75% of the organic standing stock of a first-order stream (Bilby & Likens 1980), causing flow alterations and reducing litter decomposition rates (dos Santos Fonseca *et al.* 2013). Removal of litter results in reduced retention of sediment and dissolved, fine and coarse organic materials (Bilby & Likens 1980; Bilby 1981; Webster & Tank 2000). For example, in one of the longest studies of its type (13 years), Eggert *et al*. (2012) showed that the export of gravel and fine particulate organic and inorganic matter was increased following litter exclusion.

Plant detritus also underpins food webs and promotes nutrient cycling in forest ecosystems (Moore *et al.* 2004; Hagen *et al.* 2012). Leaf litter acts as an important refuge and basal resource to organisms such as macroinvertebrates and fungi in both terrestrial (Bardgett 2005; Sayer 2006; Lavelle *et al.* 2006; Berg & McClaugherty 2008; Xu, Liu & Sayer 2013) and aquatic (Cummins & Klug 1979; Abelho 2001; Moog 2002) forest habitats. Forest biota also utilise woody debris as a substrate and energy source (Harmon *et al.* 1986), including fungi and bacteria (Tank, Webster & Benfield 1993; Tank & Winterbourn 1996; Tedersoo *et al.* 2003), invertebrates

(Anderson *et al.* 1978; Anderson & Sedelll 1979; Tank & Winterbourn 1996) and primary producers, such as macrophytes and periphyton (Cummins & Klug 1979; Eggert & Wallace 2007). This is particularly true of organisms with a low nutrient demand, or those requiring an energy source to supplement leaf material (Berg & McClaugherty 2008). In particular, the importance of twigs to invertebrates was highlighted in an exclusion experiment by Wallace *et al*. (1999), where losses of in-stream biomass and abundance of invertebrates were found when SWD was removed from a temperate forest stream. Leaves may be more important to macroinvertebrate colonisation than wood (Anderson *et al.* 1978; Hofer & Richardson 2007), although higher wood availability may increase the standing crop of xylophagous invertebrates in particular (Anderson *et al.* 1978).

While litter is clearly important to the physical and biotic components of woodlands and streams, its role is influenced by (i) the atmospheric conditions in which trees grow, and (ii) the habitat in which decay takes place. For example, atmospheric composition affects the chemical composition of both leaves (Norby *et al.* 2001) and SWD (e.g. Cotrufo & Ineson 2000). Additionally, water acidification can impact the decomposition process during freshwater breakdown (e.g. Dangles & Guérold 1998). Given the importance of litter to ecosystem functioning, it is important to understand how ongoing environmental changes will influence its decomposition. This information will allow for a better understanding of potential impacts on terrestrial and aquatic ecosystems, and can inform mitigation strategies.

Although there has been much research into the effects of environmental stressors on litter production and decay, there are still knowledge gaps to be filled. It is important to keep knowledge of these processes up-to-date, as ongoing climate change is already affecting ecosystem functioning. Specifically, this requires a better understanding at several spatial scales. For example, more work is required on the effects of multiple, potentially interacting, stressors on the processes of litter production and decay in both woodlands and streams. Equally, there is a need to identify responses at the level of biota, both in terms of chemical changes to plant litter and the direct effects of these changes on consumer organisms. While these studies have largely relied on the use of leaf litter, it is also necessary to understand

how woody material – an important component of litter budgets in woodland and stream environments – could be affected.

1.2 Thesis aims

This study investigates how environmental impacts can alter ecosystem functioning. Specifically, it considers how elevated CO_2, urban pollution and freshwater acidification affect the chemical composition and decomposition of tree litter in temperate deciduous woodlands and streams. Central aims are to examine (i) the effects of elevated CO_2 and urban pollution on the chemistry of both leaf and woody litter from deciduous trees, (ii) the responses of terrestrial and aquatic invertebrate detritivores to CO_2-treated leaf litters, and (iii) the decomposition of these litters in terrestrial and aquatic woodland environments, including acidified headwaters.

Chapter 2 reviews the literature regarding decomposition of leaf litter and small woody debris in temperate deciduous woodlands and adjacent streams, and appraises how the environmental stressors of elevated CO_2, urban pollution and acidified waters affect this process.

Most studies of invertebrate detritivore responses to elevated-CO_2 litter have focused on a small number of species, providing limited scope for identifying the responses among organisms. To help overcome this, **Chapter 3** investigates the responses of eight (four aquatic and four terrestrial) invertebrate species to leaf litter of two tree species grown under ambient and elevated CO_2. A version of this study was published in *PLOS ONE,* **9**, e86246.

Chapter 4 explores the effects of atmospheric change (increased CO_2 and urban pollution) on the chemical composition of leaf litter and subsequent effects on mass loss, nutrient dynamics and invertebrate assemblages following exposure to a temperate deciduous woodland floor.

Chapter 5 considers the themes of Chapter 4 in a freshwater context. Leaf litter chemical composition in response to different atmospheric conditions is assessed,

along with subsequent effects on mass loss, nutrient dynamics and colonisation by invertebrates and biofilms in circumneutral and acidified headwater streams.

Little work has been undertaken to investigate the effects of atmospheric change on the chemical composition and decomposition of small woody debris. **Chapter 6** seeks to address this this by investigating the effects of elevated CO_2 on the chemical composition of twigs and their breakdown on a temperate deciduous woodland floor and in streams of contrasting pH.

Chapter 7 synthesises the findings of the experimental chapters, exploring their implications and drawing general conclusions. The strengths and limitations of the experimental procedures are also highlighted, along with the remaining knowledge gaps to be investigated in future studies.

2. Literature review: The effects of multiple environmental stressors on leaf litter breakdown

2.1 Abstract

A literature review was undertaken to examine leaf and twig litter decomposition in deciduous temperate forests and headwater streams, and the effects of environmental stressors on this process. The decay of tree litter is an important ecosystem process at the foundation of detrital food web structure and function, providing a crucial step in the cycling of carbon and nutrients in both terrestrial and aquatic ecosystems. Litter decomposition advances through stages of nutrient leaching, microbial conditioning and invertebrate colonisation and maceration. This breaks coarse fragments into fine material, releasing nutrients and facilitating utilisation of litter as a substrate and food source. Litter chemical composition is an important determinant of breakdown. Higher nutritional quality (i.e. lower C/N ratio, higher nitrogen concentration and lower lignin concentration) generally leads to faster decay, as a result of increased palatability to invertebrates. Ongoing atmospheric change affects decay rates by altering litter nutritional quality: elevated CO_2 can reduce quality, whereas urban pollution can increase it. A further stressor that affects litter decomposition is that of stream acidification, where streams with low pH result in reduced decay rates due to impoverished microbial and invertebrate communities. Knowledge gaps identified in this literature review indicate that further research on litter chemical composition and decomposition is required in a number of areas, including (i) the effects of urban pollution on litter chemical quality and subsequent decay, (ii) the effects of acidification on decay, and its effects in conjunction with litter growth conditions, (iii) how the feeding responses of individual invertebrate species are affected by litter produced under elevated CO_2, and (iv) the effect of elevated CO_2 on twig chemical composition and decay, an area of study that is poorly studied in comparison with leaf litter decomposition.

2.2 Litter decomposition

2.2.1 The decomposition process

Decomposition is "the physical and chemical breakdown of detritus," a key ecosystem process in both terrestrial and aquatic environments (Chapin, Matson & Mooney 2011). It allows nutrients stored in litter to be released and subsequently cycled, which supports food webs by increasing nutrient accessibility to consumers. Terrestrial and aquatic realms are linked by the passage of litter from woodlands to adjacent streams, providing important allochthonous (externally derived) inputs of energy (Abelho 2001). This occurs via direct litterfall into streams, or via lateral entry of litter from the woodland floor. Ultimately, the process of terrestrial litter decomposition results in the mineralisation of organic matter into inorganic matter, or its transformation into complex recalcitrant compounds, with some energy lost via secondary production (Hairston Jr & Hairston Sr 1993; Nordén 1994; Chapin, Matson & Mooney 2011). In aquatic systems, coarse litter is transformed into fine particulates and dissolved organic matter that is transported downstream and utilised by stream organisms (Abelho 2001).

The process of litter decomposition advances similarly in both terrestrial and aquatic environments. Soluble compounds leach out of the litter before it is colonised and broken down by microbes, paving the way for comminution by invertebrate detritivores, breaking large fragments into progressively smaller pieces (Wagener, Oswood & Schimel 1998). Spatial progression occurs in both environments, with decomposition proceeding through 'upper' (litter layer or headwaters), 'middle' (soil-litter interface or middle reaches of a stream) and 'lower' (mineral soil or lower reaches) regions (Wagener, Oswood & Schimel 1998). The major difference may be temporal, as leaf (Treplin & Zimmer 2012) and Small Woody Debris (SWD; Sinsabaugh *et al.* 1992) decomposition tend to proceed faster in freshwater than terrestrial locations, which is likely due to the abrasive action of water on detrital surfaces (dos Santos Fonseca *et al.* 2013). This is indicated by studies that mimic stranding or re-entry of litter to streams after high flow events and confirm that aquatic episodes enhance decay rates (Hutchens & Wallace 2002; Riedl *et al.* 2013).

2.2.2 Terrestrial litter decomposition

Leaves begin leaching labile nutrients, such as tannins and other phenolics (Schofield, Hagerman & Harold 1998), when they reach the woodland floor. These soluble materials are absorbed by organisms (e.g. decomposer fungi and invertebrates), react with the soil, or are lost in solution (Chapin, Matson & Mooney 2011). Fungi and bacteria, considered primary decomposers in temperate forests (Berg & McClaugherty 2008), colonise the litter as leaching continues. Fungi are the most numerous of the microflora associated with litter, and break down the structural components of leaves by penetrating tissues and excreting digestive cellulolytic and lignolytic enzymes (Lavelle & Spain 2001; Berg & McClaugherty 2008). This causes macromolecules, such as cellulose, to break into smaller units that can be incorporated into microbial tissues. As a result, litter is physically weakened and becomes fragmented, increasing the surface area available for subsequent microbial colonisation (Chapin, Matson & Mooney 2011).

Leaching and microbial colonisation increase litter accessibility to detritivorous invertebrates, such as collembola, mites and earthworms, which contribute to the decay process by macerating large litter fragments into smaller pieces (Lavelle *et al.* 2006; Berg & McClaugherty 2008; Kampichler & Bruckner 2009). For example, *Oniscus asellus* L. has been shown to accelerate decomposition of *F. sylvatica* leaf litter and stimulate microbial respiration by 37% (Hättenschwiler & Bretscher 2001). This activity can increase total carbon and nitrogen content in leachates, affecting their availability and temporal dynamics (Huhta, Setälä & Haimi 1988; Hättenschwiler & Bretscher 2001). While maceration fragments litter, faecal pellet production also increases surface-area-to-volume ratio, further speeding microbial colonisation and incorporation into soil organic matter (Chapin, Matson & Mooney 2011). Hedde *et al*. (2007) proposed three classifications of soil litter transformers: those producing faeces with relatively high nitrogen (e.g. polydesmids and lumbricids); those producing faeces containing fine litter particles that stimulate CO_2 release (e.g. other lumbricids); and other invertebrate comminuters with weaker impacts on organic matter mineralisation.

There is limited work on the decomposition of SWD in temperate deciduous forests, given that most studies focus on leaf breakdown dynamics (Berg & McClaugherty 2008). Small woody debris breaks down more slowly than leaves due to a higher prevalence of structural components, making its decomposition more dependent on microbial enzymes for decay. One early study by Gosz *et al*. (1973) showed that SWD (0.5 cm diameter, 30–45 cm long) from the hardwoods *Betula alleghaniensis* Britton, *Acer saccharum* Marsh. and *F. grandifolia* Ehrh. did not differ in decomposition rate, but all decayed faster than the coniferous species *Picea rubens* Sarg. and *Abies balsamea* L. (Mill.) after 10 months. The size of SWD may be an important determinant of its breakdown, where twigs with 0.5 cm diameter (k = 0.055–0.081 year^{-1}) broke down faster than those with 1.5–3 cm diameter (k = 0.027–0.052 year^{-1}) in coniferous forests of the Rocky Mountains, USA (Taylor *et al.* 1991). Scheu and Schauermann (1994) found that the dimensions of *F. sylvatica* SWD also affected chemical dynamics: C/N ratio was highest in small (< 3 mm) SWD, followed by medium (3–10 mm) and then large (10 mm) SWD; C/N decreased through time, where medium and large SWD exhibited a greater loss relative to small SWD; and carbon loss was greatest in large SWD, followed by medium and then small SWD.

2.2.3 Aquatic litter decomposition

Leaf decomposition in freshwater generally occurs in three stages: leaching, conditioning and fragmentation (Petersen & Cummins 1974; Webster & Benfield 1986; Abelho 2001), although these stages are not discrete and may overlap (Gessner, Chauvet & Dobson 1999). Leaching involves the loss of labile, water-soluble molecules through purely abiotic processes, usually within the first 24 hours (Nykvist 1961). This process occurs more quickly when litter has been dried (Gessner, Chauvet & Dobson 1999). The types and amounts of compounds retained by the leaf tissue dictate subsequent colonisation and early breakdown by fungi and bacteria (Bengtsson 1992) during the aquatic conditioning phase. Fungi tend to be the most prevalent microbe in this process, particularly hyphomycetes (Suberkropp & Klug 1974; Hieber & Gessner 2002; Gessner *et al.* 2007; Krauss *et al.* 2011). As much as

10-17% of dry matter associated with litter may be composed of fungi after stream exposure (Gessner & Schwoerbel 1991; Gessner, Bärlocher & Chauvet 2003).

Invertebrate detritivores ('shredders'; Graça 2001) are particularly important litter decomposers in streams (Wallace & Webster 1996) and their presence increases decomposition rates versus microbial activity alone (Gonçalves, Graça & Callisto 2006). Shredder activity is responsible for a large proportion of leaf litter mass loss. For example, invertebrates caused 64 and 51% of overall mass loss of *Alnus glutinosa* (L.) Gaertn. and *Salix fragilis* L. leaves, respectively, in a German stream (Hieber & Gessner 2002). Leaf litter is 'prepared' for shredders by microbial conditioning, which softens and fragments leaf tissues (Graça 2001; Graça, Cressa & Gessner 2001). Despite assisting shredders, the role of fungi in the direct decomposition of litter should not be underemphasised (Gessner, Chauvet & Dobson 1999). Microbes that colonise leaf surfaces also represent a direct food source to invertebrates, given the immobilisation of leaf nutrients within their tissues (Findlay 2010). This is highlighted by the preference of the detritivores *Gammarus pulex* L. and *Sericostoma personatum* Kirby & Spence for conditioned rather than unconditioned *A. glutinosa* litter (Graça, Cressa & Gessner 2001). The identity of litter-associated fungal species may also affect shredder feeding preferences (Gonçalves *et al.* 2014). Positive feedback also occurs, as shredder excretion stimulates fungal activity by increasing local nitrogen availability (Villanueva, Albariño & Canhoto 2012).

Not all organisms use leaf litter as a food resource: some species use it as a substrate and shelter. This includes other guilds of invertebrates, including predators, grazers and filterers (Cummins & Klug 1979; Wallace & Webster 1996; Moog 2002). Wallace *et al*. (1997, 1999) showed that the abundance and biomass of invertebrates were reduced following exclusion of litter from a stream, with strong bottom-up effects from detritivores to predators. Alongside invertebrates, algae also use leaf surfaces as a substrate, forming biofilms (Hax & Golladay 1993). Algal colonisation can result in greater palatability to invertebrate detritivores (Franken *et al.* 2005), leading to faster decay rates (Rier, Kuehn & Francoeur 2007; Danger *et al.* 2013).

Aside from leaf material, woody litter also enters and decays in freshwater environments. There are some similarities between leaf and twig decay in streams.

For example, SWD is also broken down by stream organisms, including fungi (Shearer & Webster 1991), although microbial respiration was an order of magnitude lower on small wood (less than 40 mm diameter) compared to leaf litter in a headwater stream in the Appalachian Mountains, USA (Gulis, Suberkropp & Rosemond 2008). Saproxylophagous invertebrates are also associated with wood (Moog 2002) and have been shown to affect aquatic SWD breakdown in conifer forests (Anderson *et al.* 1978). Webster *et al*. (1999) synthesised data from the Coweeta Hydrologic Laboratory, North Carolina, and found that the breakdown rate of sticks (< 3 cm diameter) was much lower than for leaves. Similarly, *A. rubra* Bong. leaves lost around 50% of their mass compared to less than 15% mass loss in *A. rubra* wood veneers (Hofer & Richardson 2007). A review by Spänhoff and Meyer (2004) found that natural SWD breaks down slowly in freshwaters, with typical decay rates (k) ranging from 0.02 to 0.45 year^{-1}.

Commercially-modified wood substrates (e.g. veneer strips, ice cream sticks and wood chips) have generally larger surface-area-to-volume ratios than natural products and break down faster, with decay rates (k) of 0.10 to 3.1 year^{-1}. More recent work by Aristi *et al*. (2012) showed large differences (k = 0.12–6 year^{-1}) in the breakdown of *P. nigra* × *canadinensis* tongue depressors across a range of rivers with differing physicochemical, biological and geomorphological features. Similarly, it is known that water chemistry (Díez *et al.* 2002; Gulis *et al.* 2004), stream order (Melillo *et al.* 1983; Díez *et al.* 2002), tree species (Webster *et al.* 1999; Díez *et al.* 2002; Spänhoff & Meyer 2004) and the presence of decomposing leaf material (Webster & Tank 2000) are all factors that influence the breakdown of woody debris in streams. Factors such as altitude, catchment area, toxicity and riparian buffer width have also been implicated in the decay of tongue depressors (Aristi *et al.* 2012).

2.2.4 Chemical control of breakdown

One particularly important factor influencing the decomposition of leaves and SWD in terrestrial and aquatic environments is that of litter chemical composition. Freschet *et al*. (2012) found that the terrestrial decomposability of a range of plant species and tissues appears to be controlled by lignin, carbon and dry matter content, while

nutrient-related traits such as nitrogen and phosphorus content can have a more variable effect. Other terrestrial studies have found that high concentrations of nitrogen and phosphorus, as well as lower C/N ratios and lignin concentrations, are linked to faster decay in woodland settings (Melillo, Aber & Muratore 1982; Zhang *et al.* 2008). This general outcome extends into freshwaters, where lower phosphorus, and increased lignin and cellulose, were correlated with lower decomposition rates in a study of *A. glutinosa* (Lecerf & Chauvet 2008).

A global meta-analysis of terrestrial wood decomposition found that nitrogen, phosphorus and C/N ratio correlate with angiosperm decomposition rates, which could be due to a direct effect on decomposer activity, or an indirect effect on the microsite in which decomposition is taking place (Weedon *et al.* 2009). Wood chips of five tree species with high lignin/N ratios broke down more slowly in low-order streams, while high lignin content resulted in slower breakdown in high-order streams (Melillo *et al.* 1983). The activity of lignocellulose-degrading enzymes was also positively associated with decomposition of *B. papyrifera* Marsh. ice-cream sticks, further demonstrating the importance of lignin to SWD decay (Sinsabaugh *et al.* 1992). In another study, pine branches (3 cm diameter, 10 cm long) contained lower nitrogen and phosphorus than alder and oak, and also broke down more slowly (Díez *et al.* 2002).

Regardless of habitat, high concentrations of structural polymers (e.g. lignin and celluloses) increase the physical toughness of litter, resulting in greater resistance to both biotic and abiotic factors. Many invertebrates prefer litter with high nutrient concentrations and a low C/N ratio (Anderson & Sedelll 1979; Cummins & Klug 1979), finding it difficult to digest litter with high structural and defensive (e.g. tannins and secondary chemicals) content, which reduce the overall nutritional quality of leaf (Graça, Cressa & Gessner 2001; Motomori, Mitsuhashi & Nakano 2001) and wood (Cornwell *et al.* 2009) litter.

2.3 Atmospheric change

Anthropogenic activities, such as the burning of fossil fuels, have increased atmospheric greenhouse gas concentrations by 40% since pre-industrial times (IPCC 2013). Greenhouse gas molecules – including carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gases (F-gases) – contribute to the 'greenhouse effect': a net global temperature increase due to absorbance and re-radiation of solar energy to the Earth's surface (Houghton 2009). Ongoing land-use change, urbanisation and industrial activity threaten to exacerbate the problem (Akimoto 2003; Karl & Trenberth 2003).

According to data collected by the National Oceanic and Atmospheric Administration (NOAA) at Mauna Loa, Hawaii, the mean atmospheric concentration of CO_2 for 2013 (the latest full year on record) was 396.5 parts per million (ppm), with an average annual increase of 2.1 ppm $year^{-1}$ (Tans & Keeling 2014). Ice-core data suggest that global atmospheric CO_2 has not risen above this concentration for the last 800,000 years (Lüthi *et al.* 2008), or perhaps for 15 million years (Tripati, Roberts & Eagle 2009). Anthropogenic activity is related to these changes, with over 75% of fossil fuel emissions involving the release of this molecule (IPCC 2013). The latest report by the Intergovernmental Panel on Climate Change (IPCC) incorporated a range of atmospheric CO_2 projections based on the predicted development of factors including global population, economics and technology. For example, one scenario that assumes increasing greenhouse gas emissions through time (known as RCP 8.5) predicts that atmospheric CO_2 concentrations could more than double, reaching as much as 1000 ppm in the next 100 years.

2.3.1 Effects of atmospheric CO_2 on litter

Areas with naturally or artificially increased CO_2 concentrations are used to study the effects of elevated CO_2 on tree litter chemical quality. Some research has taken place in locations with naturally high CO_2 concentrations, such as CO_2 springs (Hättenschwiler *et al.* 1997), but these are rare and concentrations cannot be adjusted. Most studies have controlled CO_2 concentrations using one of several methods

(Ceulemans & Mousseau 1994; Saxe, Ellsworth & Heath 1998), each of which have associated advantages and disadvantages. For example, it is relatively inexpensive to construct and maintain open- or closed-top chambers, although such designs can also affect the local microclimate, and are generally suitable for immature potted plants only. Outdoor techniques such Free-Air Carbon Enrichment (FACE) may give the most accurate predictions of long-term plant responses to elevated CO_2, as a large number of mature trees can be grown for years under field conditions (Lewin *et al.* 1994). Initial outlay and maintenance are, however, relatively higher for FACE facilities, and it may be more difficult to maintain consistent gas concentrations (Saxe, Ellsworth & Heath 1998).

2.3.2 Effects of elevated CO_2 on litter chemical composition

Elevated atmospheric CO_2 alters the chemical composition of leaves while still on the parent tree. Reduced leaf nitrogen concentration is typical of plants grown under elevated CO_2, with a meta-analysis by Cotrufo *et al*. (1998) indicating an average reduction of 14%, with greater losses for C3 than C4 and nitrogen-fixing plants. Coûteaux *et al*. (1999) found that these effects were species-specific and dependent on the length of CO_2 exposure. Taub and Wang (2008) suggested possible reasons for low nitrogen concentrations following high CO_2 exposure: (i) dilution due to increased carbon assimilation, (ii) reduced uptake rates due to reductions in demand or the ability of the soil-root system to supply it, (iii) reduced transpiration due to reduced stomatal conductance, and (iv) increased losses as volatiles or through root exudates. There may also be a dilution effect associated with increases in non-structural carbohydrate production per unit leaf area (Ainsworth & Long 2005). Nitrogen limitation may not always be a problem, however, as species such as *A. glutinosa* have the ability to fix nitrogen via root nodules, which may increase in size under elevated CO_2 to allow for relatively more nitrogen to be fixed (Temperton *et al.* 2003). While nitrogen concentration decreases, increased photosynthetic rates and fixation of CO_2 (Ainsworth & Long 2005; Ainsworth & Rogers 2007) can increase carbon concentration. This results in a larger C/N ratio (Lindroth 2010), with a study by Gifford *et al*. (2000) indicating an increase of approximately 15% under doubled CO_2.

Carbon dioxide enrichment changes the chemical composition of leaf litter across a range of tree species and growing conditions. In general, nitrogen concentration is reduced, with increases in C/N ratio, and concentrations of lignin and phenolics (Norby *et al.* 2001; Tuchman *et al.* 2003b; Parsons, Lindroth & Bockheim 2004; Oksanen *et al.* 2005). Results are, however, dependent on growth environment (open-top chamber, solardome or no chamber) and whether the plant was grown in a pot. These results mean that differences in chemical composition between ambient- and elevated-CO_2 leaves are maintained after falling as litter. The magnitude of the difference may be enhanced, as for C/N ratio (Tuchman *et al.* 2002), or diminished, as for nitrogen concentration (Norby *et al.* 2001). Relative amounts of chemicals may also be affected by senescence. For example, one study has shown that elevated-CO_2 leaves and litter both contained a higher concentration of phenolics than ambient-CO_2 material, but senescence had halved the concentration (Tuchman *et al.* 2002). The study also found no difference in the lignin concentration of green leaves grown under ambient and elevated CO_2, but the latter had a significantly higher concentration after senescence.

Relatively little work has been undertaken on the decomposition of deciduous SWD produced under elevated CO_2. In one study, elevated CO_2 (350, 500 or 750 ppm) induced little change in the nitrogen and phosphorus content of woody tissues sourced from saplings of multiple temperate deciduous species (Williams *et al.* 1986). Chemical changes to stem wood have, however, been recorded under elevated CO_2. The lignin content of *P. tremula* × *alba* Aiton (Sm.) increased under elevated CO_2 (chambers, 800 ppm) as a result of increased carbon supply to the stem and subsequent enhancement to the process of lignin synthesis (Richet *et al.* 2012). Carbon dioxide enrichment (greenhouses, 700 ppm) also reduced nitrogen concentrations in woody material of *Castanea sativa* Mill., but a concurrent increase in biomass resulted in no difference in total tree nitrogen (El Kohen, Rouhier & Mousseau 1992). Kostiainen *et al*. (2006) grew seven-year old *B. pendula* Roth trees under elevated CO_2 (open-top chambers, 2 × ambient) for three growing seasons in open-top chambers, resulting in decreased levels of cellulose and lignin. Over a longer period of time (five growing seasons) and in a FACE facility, CO_2 enrichment (560 ppm) affected the chemical composition of *Populus tremuloides* Michx. and *B.*

papyrifera trees (Kostiainen *et al.* 2008), with increased uronic acids in aspen, and decreased starch in birch. Aside from leaves, responses of wood chemical composition to elevated CO_2 may be species- and clone-specific, as Kaakinen *et al*. (2004) found increases in soluble sugar concentration in one *P. tremuloides* clone under elevated CO_2 (FACE, 560 ppm) and decreased starch concentration in two clones, while hemicellulose concentration in *B. papyrifera* was decreased and little response was found in *A. saccharum*.

2.3.3 Linking elevated CO_2, litter chemical composition and breakdown

Few studies have been undertaken to uncover how litter decay is affected by chemical composition changes as mediated by CO_2 enrichment. Studies that have been undertaken show that mass loss proceeds at a slower rate for elevated-CO_2 litter (Table 2.1). In general, this is linked to a reduced nitrogen concentration, along with an increased C/N ratio and lignin concentration (Table 2.1), although increased condensed tannins (Ostrofsky 1997) and total phenolic compounds (Tuchman *et al.* 2003a; b) have also been found in conjunction with slower decay. Chemical dynamics are affected by CO_2 treatment and species. For example, Rier *et al*. (2005) found that *P. tremuloides*, *S. alba* Kern. and *A. saccharum* litters produced under elevated atmospheric CO_2 (open-top chambers, 720 ppm) had increased soluble phenols, carbohydrate-bound condensed tannins and C/N ratios than litter grown under ambient CO_2, although effects were species-specific. Following this, exposure to a northern hardwood forest stream showed that litter C/N ratios generally declined through time, although elevated-CO_2 litter generally had a higher C/N ratio than that of ambient-CO_2 litter over 80 days.

Little work has been conducted on the breakdown of SWD following growth under altered atmospheres. Cotrufo and Ineson (2000) grew *F. sylvatica* twigs (2 cm diameter) under elevated CO_2 (open-top chambers, ambient + 350 ppm), resulting in 38% lower nitrogen and 12% lower lignin than ambient-CO_2 twigs, and subsequently higher C/N and lignin/N ratios. These chemical changes did not result in slower terrestrial decomposition, however, nor was there an effect on nitrogen and lignin dynamics through time. Twigs (1–2 mm diameter) of *P. abies* (L.) H. Karst. grown

under elevated CO_2 (open-top chambers, 550 ppm) had lower nitrogen concentrations, but did not differ in carbon or lignin concentrations, when compared to twigs grown under ambient CO_2 (Hättenschwiler, Bühler & Körner 1999). This resulted in slower decomposition of elevated-CO_2 twigs, with 26% mass lost over 331 days of incubation in a temperate forest, compared to 50% mass loss for ambient-CO_2 twigs.

2.3.4 Invertebrate responses to elevated-CO_2 litter

Changes to chemical composition caused by atmospheric CO_2 enrichment can affect invertebrate feeding and life histories. This has been established for green leaf tissues and herbivores, where leaf-chewing insects compensated for reduced nutritional quality by consuming more material, while phloem feeders increased population sizes and reduced development times (Bezemer & Jones 1998). After falling as litter, elevated-CO_2 leaves continue to affect the feeding behaviour of invertebrate consumers. When given a direct choice between common ash (*Fraxinus excelsior* L.) litter grown in ambient and enriched CO_2, the terrestrial isopod detritivore *O. asellus* consumed less of the treated material (Cotrufo, Briones & Ineson 1998). These findings suggest a preference for litter of higher nutritional quality, since litter grown under ambient conditions contains more nitrogen. When fed with either treated or reference litter from common beech (*F. sylvatica*) in a no-choice scenario, *O. asellus* and another detritivorous isopod, *Porcellio scaber* Latreille, consumed more of the former (Hättenschwiler & Bretscher 2001). This result suggests that a compensatory feeding response is elicited when isopods are presented with material of lower nutritional quality. In a choice between *A. pseudoplatanus* L., *F. sylvatica* and *Quercus robur* L. grown at ambient or elevated CO_2, *O. asellus* preferred *A. pseudoplatanus* to *F. sylvatica*, but only when all litter was produced at elevated CO_2 (Hättenschwiler & Bretscher 2001). These results may not always be the case: CO_2 enrichment did not result in changes to *Porcellio* species across a range of hardwoods, although there were differences in breakdown rates as a result (Cotrufo, Drake & Ehleringer 2005).

Table 2.1. Effects of elevated CO_2 on litter chemistry and subsequent mass loss, relative to effects of ambient CO_2 (S = Solardomes; FACE = Free-Air Carbon Enrichment; OTC = Open-Top Chambers)

Study	Elevated CO_2 (delivery system)	Species	Decay site (duration)	Mass loss	C	N	C/N	Lignin
Cotrufo, Ineson & Rowland (1994)	600 ppm (S)	*Fraxinus excelsior* L., *Betula. Pubescens* Ehrh., *Acer pseudoplatanus* L.	Terrestrial microcosm (155 days)	Slower	Lower		Higher	Higher
Cotrufo & Ineson (1996)	Ambient + 250 ppm (S)	*B. pendula* Roth	Terrestrial (1 year)	Slower			Higher	Higher
Cotruo, Briones & Ineson (1998)	600 ppm (S)	*F. excelsior, A. pseudoplatanus*	Terrestrial (1 year)	Slower				
Cotrufo, De Angelis & Polle (2005)	2 × ambient (FACE)	*Populus* spp.	Terrestrial (8 months)	Slower		Lower		No change
Hättenschwiler, Bühler & Körner (1999)	550 ppm (OTC)	*Fagus sylvatica* L.	Terrestrial (331 days)	Slower	No change	Lower		No change
Ostrofsky (1997)		48 deciduous spp.	Aquatic	Slower		Lower	Higher	Higher
Tuchman *et al*. (2003b)	720 ppm	*P. tremuloides* Michx.	Aquatic (120 days)	Slower		Lower	Higher	Higher

Altered leaf chemistry as a result of CO_2 enrichment can also affect litter nutritional quality for stream food webs (Tuchman *et al.* 2003b), altering shredder feeding behaviour. For example, when presented with a choice between litter grown in ambient or elevated CO_2 conditions, larvae of the caddis fly *S. vittatum* Rambur preferred the latter (Ferreira *et al.* 2010). It is not simply consumption that is affected: changes in leaf chemistry brought about by enriched CO_2 cause the crustaceans *G. pulex* and *Asellus aquaticus* L. to excrete more nitrogen and phosphorus than when fed on ambient material (Frost & Tuchman 2005). This loss of nutrients may be partly responsible for effects such as reduced growth and increased mortality of invertebrates (Tuchman *et al.* 2002, 2003b). Further investigation is required to understand the responses of shredding macroinvertebrates to food quality that has been altered by climate change processes.

2.3.5 Effects of atmospheric pollution on litter

Air pollution is known to affect plants physically. For example, photosynthesis can be affected by stomatal closure induced by air pollution, reducing carbon accumulation (Darrall 1989). When scaled-up, forests can act as a sink for air contamination when pollution levels are low, but tree mortality is the likely outcome for prolonged exposure to high levels of pollution (Smith 1974). Such forest declines can be linked to reduced soil quality caused by increased deposition of sulphur, nitrate and ammonium (Schulze 1989).

There has been relatively little work on the effects of urban pollution on litter chemistry and decay. Concentrations of airborne pollutants (e.g. NO^{3-}, NH_4^{+}) are normally higher in urban than rural locations (George *et al.* 2007). This can result in increased soil nitrogen deposition may result in greater nitrogen availability to trees (Lovett *et al.* 2000; Zhu & Carreiro 2004; Fang *et al.* 2011), but outcomes for litter nutritional quality appear unpredictable (Pavao-Zuckerman & Coleman 2005). Increased concentrations of lignin and labile materials have, however, been identified in urban litter (Carreiro *et al.* 1999). This may result in slower decomposition rates relative to rural litter when decaying in terrestrial forest environments (Carreiro *et al.* 1999; Pouyat & Carreiro 2003).

2.4 Effects of stream acidification on litter

Fossil fuel consumption has changed the chemical quality of freshwaters by altering the composition of atmospheric gases. Atmospheric concentrations of pollutant gases such as sulphur and nitrogen oxides have increased, resulting in greater absorption into rainwater to create acid rain. This effect has perhaps been most damaging in Europe and North America, where acid rain has combined with base-poor soils to lower the pH of runoff from land to water, increasing the aquatic concentration of metals such as aluminium to harmful levels (Schindler 1988). The problem of acid rain peaked in the 1970s, but acidification still occurs episodically or even chronically, and still poses a threat to freshwater environments across the globe (Kowalik *et al.* 2007; Ormerod & Durance 2009).

Stream acidification has consistently been shown to retard the decomposition of organic matter. The Fernow Whole-Watershed Acidification Experiment in West Virginia, USA (Adams *et al.* 1993), simulated the effects of acid deposition by adding ammonium sulphate fertiliser to streams. At this site, Adams and Angradi (1996) recorded slower decay of *Liriodendron tulipifera* L., *Prunus serotina* Ehrh. and *B. lenta* L. litter in an acidified watershed compared to an untreated one, while litter of *A. saccharum* and *Q. alba* L. broke down slowest at pH 4.3 and fastest at pH 6.0, with an intermediate decay rate at pH 7.5 (Griffith & Perry 1994). Away from Fernow, Dangles *et al*. (2004) observed the breakdown of *F. sylvatica* leaves in 25 French streams across a pH gradient, with decay proceeding up to 20 times slower in the most acidified and aluminium-rich site compared to locations with more neutral pH. This confirmed earlier work by Dangles and Guérold (1998, 2001) demonstrating slower breakdown of *F. sylvatica* litter in acidified streams compared to circumneutral streams over seven and eight month exposure periods, respectively.

Slower breakdown of litter in acidified streams may be due to reduced biotic activity. For example, fungal biomass and activity have been shown to decrease after exposure to acidified waters (Griffith & Perry 1994; Dangles *et al.* 2004), which could directly slow decomposition rates. The composition of primary producers, particularly diatoms, also changes substantially at low pH (Hirst *et al.* 2004). Since fungal (Bärlocher 1985; Graça 2001) and algal (Rier, Kuehn & Francoeur 2007; Danger *et al.*

2013) colonisation of litter can increase palatability to invertebrates, stream acidification could indirectly reduce shredder impacts by reducing their biomass. Water acidity also acts directly on invertebrates, with different species expressing various sensitivities (Moog 2002). This can result in impoverished invertebrate communities (Mackay & Kersey 1985; Simpson, Bode & Colquhoun 1985; Sutcliffe & Hildrew 1989) and reduced numbers of shredders (Dangles 2002) at acidified sites. For example, Dangles and Guérold (1998, 2001) found that the acid sensitive amphipod *G. fossarum* Koch was the most dominant shredder at non-acidified sites, but *Leuctra* and *Protonemura* plecopterans were the most abundant at acid sites. This could explain the reduced decomposition rate in the acid stream, as *G. fossarum* is a more efficient shredder than *Leuctra* or *Protonemura* species.

Acidification remains a threat to stream functioning despite some signs of recovery. This is clear from work at Llyn Brianne Stream Observatory, Wales, UK, which hosts one of the longest running investigations into the effects of land use and acid deposition on freshwater environments. An assessment of data stretching back 25 years by Ormerod and Durance (2009) indicated that recovery from acidification is occurring at the site, but this has not resulted in full recovery of invertebrate species. Ongoing acidic episodes at the site appear to explain this slow biological recovery (Kowalik *et al.* 2007). To investigate this, Pye *et al*. (2012) simulated episodic acidification by transplanting *Q. robur* litter bags between acidified and circumneutral streams. This reduced decomposition rates and suppressed acid-sensitive families of Plecoptera, the major colonists of litter in the study. Experimental liming has indicated that the effects of acidified waters on decomposition can be reversed: in the Wye Valley, UK, *F. sylvatica* decayed slower in acidified versus circumneutral streams, but experimental aerial liming resulted in a slight increase in pH and a decay rate indistinguishable from that found in circumneutral sites (Merrix, Lewis & Ormerod 2006). There is some doubt, however, that this approach is more effective than natural recovery (Ormerod & Durance 2009).

2.5 Conclusions

Litter decomposition is a central process to woodland ecosystem functioning, influencing nutrient cycling, carbon storage and food web structure. Atmospheric change as a result of fossil fuel combustion could compromise this process by altering the chemical composition of tree leaf litter and its subsequent decay in both terrestrial (woodland floor) and aquatic (stream) ecosystems. In addition, acid rain and runoff from polluted soils have both contributed to the acidification of freshwaters, delaying the decomposition process further. Several areas are highlighted as requiring the need for greater understanding: (i) the effects of rural and urban locations on litter chemical composition and subsequent decomposition, (ii) effects of acidification in combination with effects of atmospheric pollution on litter chemical composition and decomposition, (iii) a more comprehensive study of invertebrate feeding responses to litter with chemical composition altered by elevated CO_2, and (iv) the effect of elevated CO_2 on the chemical composition and decomposition of small woody debris in terrestrial and aquatic habitats. These issues must be addressed to achieve a greater understanding of the effects of ongoing global change processes on the functioning of both terrestrial and aquatic ecosystems, allowing for a greater ability to predict and mitigate against potentially harmful ecosystem changes.

3. Effects of elevated CO_2 on litter chemistry and subsequent invertebrate detritivore feeding responses

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3.1 Abstract

Elevated atmospheric CO_2 can change foliar tissue chemistry. This alters leaf litter palatability to macroinvertebrate detritivores with consequences for decomposition, nutrient turnover, and food-web structure. Currently there is no consensus on the link between CO_2 enrichment, litter chemistry, and macroinvertebrate-mediated leaf decomposition. To identify any unifying mechanisms, eight invertebrate species from aquatic and terrestrial ecosystems were presented with litter from *Alnus glutinosa* (common alder) or *Betula pendula* (silver birch) trees propagated under ambient (380 ppm) or elevated (ambient + 200 ppm) CO_2 concentrations. Alder litter was largely unaffected by CO_2 enrichment, but birch litter from leaves grown under elevated CO_2 had reduced nitrogen concentrations and greater C/N ratios. Invertebrates were provided individually with either (i) two litter discs, one of each CO_2 treatment ('choice'), or (ii) one litter disc of each CO_2 treatment alone ('no-choice'). Consumption was recorded. Only *Odontocerum albicorne* showed a feeding preference in the choice test, consuming more ambient- than elevated-CO_2 birch litter. Species' responses to alder were highly idiosyncratic in the no-choice test: *Gammarus pulex* and *O. albicorne* consumed more elevated- than ambient-CO_2 litter, indicating compensatory feeding, while *Oniscus asellus* consumed more of the ambient-CO_2 litter. No species responded to CO_2 treatment when fed birch litter. Overall, these results show how elevated atmospheric CO_2 can alter litter chemistry, affecting invertebrate feeding behaviour in species-specific ways. The data highlight the need for greater species-level information when predicting changes to detrital processing – a key ecosystem function – under atmospheric change.

3.2 Introduction

Global concentrations of atmospheric carbon dioxide (CO_2) could more than double by 2100 (Collins *et al.* 2013). Typically, CO_2 enrichment leads to increased plant photosynthesis, resulting in greater biomass and production (Curtis & Wang 1998). Plant tissue chemistry is typically modified, with decreasing nitrogen concentrations and increasing carbon-nitrogen (C/N) ratios affecting herbivore life-history and feeding responses (Robinson, Ryan & Newman 2012).

Approximately 90% of primary production in forest ecosystems escapes herbivory and forms detritus (Cebrian 1999), providing a crucial energy pool that underpins the trophic structure of soils and adjacent freshwaters (Moore *et al.* 2004). The effect of elevated CO_2 on the chemical composition of green foliar tissues reduces its palatability to detritivores when it falls as litter (Tuchman *et al.* 2002). In particular, elevated CO_2 can reduce litter resource quality by decreasing litter nitrogen content (Coûteaux *et al.* 1999; Norby *et al.* 2001), subsequently increasing C/N ratios (Cotrufo, Ineson & Rowland 1994; Tuchman *et al.* 2003b). Increases in structural (Cotrufo, Ineson & Rowland 1994; Norby *et al.* 2001; Tuchman *et al.* 2002) and defensive (Tuchman *et al.* 2003b; Parsons, Lindroth & Bockheim 2004) compounds have also been reported, along with both increases and decreases in phosphorus concentrations (Liu, King & Giardina 2007; Ferreira *et al.* 2010). The potential for rising CO_2 concentrations to alter litter chemical composition is established, but the consequences for invertebrate-mediated decomposition – an important ecosystem function – remain unclear (Prather *et al.* 2013).

Detritivorous macroinvertebrates are functionally important in detritus-based ecosystems (Yang & Gratton 2014), as they are responsible for both comminution and consumption of litter, releasing nutrients for other organisms, such as saprophagous fungi (Wallace & Webster 1996; Lavelle *et al.* 2006). To maintain optimal body nutrient concentrations, theoretical predictions and empirical evidence suggest that invertebrates can increase feeding rates of reduced-quality material (e.g. Cotrufo, Briones & Ineson 1998; Hättenschwiler, Bühler & Körner 1999), a process known as ‘compensatory feeding’ (as defined by Gessner *et al.* 2010). Despite this, poor quality

litter has also been shown to increase handling times (Ott, Rall & Brose 2012), while reducing nutrient assimilation, slowing development rates, and increasing mortality (Tuchman *et al.* 2002; Frost & Tuchman 2005). These conflicting responses have resulted from studies focusing on a small number of species (e.g. Hättenschwiler, Bühler & Körner 1999; Ferreira *et al.* 2010), which also fail to incorporate aquatic and terrestrial invertebrates, despite differences in detrital accumulation and energy flow between these habitats (Shurin, Gruner & Hillebrand 2006). A broad-scale study incorporating a range of invertebrate species from different habitats is essential to identify the unifying mechanisms that govern invertebrate feeding responses to elevated-CO_2 litter.

In this study, the feeding preferences and consumption rates of eight detritivorous macroinvertebrate species presented with *Alnus glutinosa* (L.) Gaertn. (common alder) and *Betula pendula* Roth (silver birch) leaf litter produced under ambient and elevated atmospheric CO_2. It was hypothesised that: (1) CO_2 enrichment will reduce leaf chemical quality and, given nitrogen-fixing ability in alder, responses will differ by tree species; (2) when presented with a choice between ambient- and elevated-CO_2 litter, invertebrates will prefer ambient material, assuming its higher quality; (3) when given litter of one CO_2 treatment only, consumption of elevated-CO_2 litter will be greater, to compensate for its reduced quality.

3.3 Materials and methods

3.3.1 Leaf litter preparation

Alder and birch litters were produced at the BangorFACE facility, Bangor, UK (Smith *et al.* 2013; Fig. 3.1). Trees were grown in eight identical plots (four ambient CO_2 and four elevated CO_2) to minimise infrastructure-induced artefacts. CO_2 enrichment was carried out using high velocity pure CO_2 injection, controlled using equipment and software modified from EuroFACE (Miglietta *et al.* 2001). Elevated CO_2 concentrations, measured at 1 min intervals, were within 30% deviation from the pre-set target concentration of 580 ppm CO_2 (ambient + 200 ppm) for 75–79% of the photosynthetically-active period (daylight hours from budburst until leaf abscission)

of 2005–2008. Vertical profiles of CO_2 concentration measured at 50 cm intervals through the canopy showed a maximum difference of +7% from reference values obtained at the top of the canopy (Smith *et al.* 2013). From the beginning of leaf senescence, fallen leaf litter was collected weekly until all leaves had abscised (October to December). Litter within each CO_2 treatment was homogenised and air-dried.

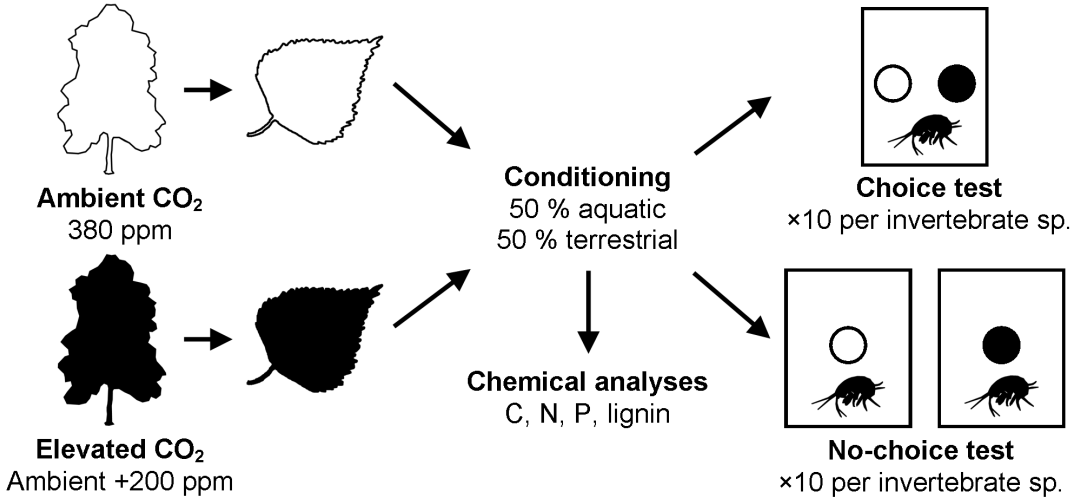

Fig. 3.1. Overview of the experimental approach. Litter was produced under ambient and elevated CO_2 atmospheres at BangorFACE, UK. Half of the litter from each CO_2 treatment was conditioned aquatically and half terrestrially. Chemical analyses of the conditioned litter were undertaken, and litter discs were presented to aquatic and terrestrial invertebrates in choice and no-choice tests. Only one tree and one invertebrate species have been shown for clarity. Not to scale.

Initial chemical leaching and microbial colonisation of litter ('conditioning') are crucial steps in making litter palatable to detritivorous macroinvertebrates (Daniel *et al.* 1997; Graça, Cressa & Gessner 2001). Prior to the start of the experiment, litter was conditioned in fine mesh bags (100 µm to permit microorganisms only) placed in plastic containers (29 × 29 × 10 cm; Fig. 3.1). For each tree species × CO_2 treatment combination, one bag was placed in aerated stream water that was inoculated with stream-collected litter of mixed-species origin ('aquatic conditioning'); a second bag per tree species × CO_2 treatment combination was inserted between field-collected soil and mixed deciduous leaf litter ('terrestrial conditioning'). Containers were maintained at 11 ± 1°C with a 12:12 h light-dark cycle and terrestrial containers were

sprayed with deionised water every three days to maintain humidity (approximately 50%). These conditions were selected to represent natural conditioning processes in aquatic and terrestrial habitats in a controlled manner. After two weeks, leaf discs were cut using a 9 mm diameter cork-borer (avoiding the mid-vein), which were air-dried and weighed (± 0.1 mg) prior to experimental use.

Litter samples allocated to chemical analyses (Fig. 3.1) were stored at –80°C before being oven-dried (50°C for 24 h) and ground into powder (120 s, 50 beats s^{-1}; Pulverisette 23 ball mill, Fritsch GmbH, Idar-Oberstein, Germany). Each sample was composed of litter from three separate leaves. For carbon, nitrogen and phosphorus analyses, five samples were processed per tree species × CO_2 treatment × conditioning type combination; for lignin analysis, four samples were used. The percentage leaf dry mass (% leaf DM) of carbon and nitrogen, and the Carbon-Nitrogen (C/N) ratio, were determined by flash combustion and chromatographic separation of approximately 1.5 mg leaf powder using an elemental analyser (Elemental Combustion System 4010 CHNS-O Analyzer, Costech Analytical Technologies, Inc., Milan, Italy), calibrated against a standard ($C_{26}H_{26}N_2O_2S$). Phosphorus concentrations (% leaf DM) were quantified using X-ray fluorescence (see Reidinger, Ramsey & Hartley 2012 for detailed methodology). The percentage of acetyl-bromide-soluble lignin in litter Dry Cell Walls (% DCW) was determined following the acetyl bromide spectrophotometric method (Foster, Martin & Pauly 2010). Lignin-Nitrogen (lignin/N) ratios were calculated for each tree species × CO_2 treatment × conditioning treatment combination.

3.3.2 Invertebrates

Eight macroinvertebrate species were selected for study (Table 3.1), representing a taxonomic range of litter consumers found in temperate forest habitats (Moog 2002; Wurst, De Deyn & Orwin 2012). Aquatic species were collected from streams in the Brecon Beacons National Park, South Wales, UK (51°50’53” N, 3°22’16” W and 51°50’55” N, 3°33’43” W) and Roath Park, Cardiff, UK (51°30’00” N, 3°10’10” W); terrestrial species were collected from soil-litter interfaces in Bute Park, Cardiff, UK (51°48’49” N, 3°18’24” W). All individuals were adults, apart from larval

Odontocerum albicorne and *Sericostoma personatum* caddis flies. Individuals from within each species were selected for size similarity. Prior to experimental use, invertebrates were maintained for at least four weeks in single-species containers (11 ± 1°C, 12:12 h light-dark cycle) and were fed *Fagus sylvatica* L. (common beech) litter conditioned as for experimental litter, preventing habituation to experimental alder and birch litter. Feeding was ceased two days prior to the experiments to allow for gut clearance.

3.3.3 Experimental arenas

All experiments were conducted in 11 × 16.5 × 3.5 cm lidded plastic arenas (Cater For You Ltd, High Wycombe, UK) lined with compacted sterilised aquarium gravel (Unipac, Northampton, UK) and were maintained at 11 ± 1°C with a 12:12 h light-dark cycle. Aquatic microcosms were filled with 400 ml of filtered (100 µm mesh) stream water (circumneutral pH; collected from 51°50'53" N, 3°22'16" W) and aerated through a pipette tip (200 µl Greiner Bio-One) attached to an air-line. Terrestrial microcosms were sprayed with deionised water every three days to maintain moisture content and humidity (approximately 50%). All arenas were uniquely labeled ('microcosm ID'). These standardised conditions were chosen to mimic natural habitats, while minimising the availability of supplementary organic material that could act as a confounding resource during the feeding trials.

Table 3.1. Detritivorous macroinvertebrate species used in the study.

Habitat	Name	Authority	Order: Family
Aquatic	*Asellus aquaticus*	(Linnaeus 1758)	Isopoda: Asellidae
	Gammarus pulex	(Linnaeus 1758)	Amphipoda: Gammaridae
	Odontocerum albicorne	(Scopoli 1763)	Trichoptera: Odontoceridae
	Sericostoma personatum	(Kirby & Spence 1826)	Trichoptera: Sericostomatidae
Terrestrial	*Blaniulus guttulatus*	(Bosc 1792)	Julida: Blaniulidae
	Oniscus asellus	Linnaeus 1758	Isopoda: Oniscidae
	Porcellio scaber	Latreille 1804	Isopoda: Porcellionidae
	Tachypodoiulus niger	(Leach 1815)	Julida: Julidae

For litter of each tree species, detritivores were presented with: (i) a choice between ambient- and elevated-CO_2 material, to provide a direct comparison of detritivore preferences, and (ii) a no-choice situation with each CO_2 treatment presented on its own, approximating litter consumption in current (ambient CO_2) and future (elevated CO_2) atmospheric conditions (Fig. 3.1). In each experiment, ten microcosms were set up for each invertebrate and tree species combination (n = 160). A single invertebrate was added to each arena and was placed in the end opposite the airline in aquatic arenas and equidistant to both discs in the choice test. In the choice test, one disc of each CO_2 treatment was pinned to the centre of the arena, 4 cm apart. Discs were replenished when at least 50% of the existing disc had been consumed. In the no-choice test, half of the microcosms contained one ambient-CO_2 disc and the other half one elevated-CO_2 disc, pinned to the centre of the arena. Both experiments ended after 14 days, or when five (50%) of the individuals of a specific species consumed at least 50% of one disc (choice experiment only). For each invertebrate, the total mass of litter consumed was calculated (± 0.1 mg). For choice experiment data, this value was divided by the number of days over which the test had taken place.

Additionally, control microcosms were set up to ensure that differences in mass loss between CO_2 treatments were due to invertebrate activity alone. For each experiment, ten microcosms were set up for each habitat type × tree species combination. Controls for the choice test each contained one disc of each CO_2 treatment; half of the no-choice control microcosms contained one ambient-CO_2 disc and the other half contained one elevated-CO_2 disc. Leaf discs were air-dried and weighed (± 0.1 mg) after 14 days and their total mass loss calculated.

3.3.4 Data analysis

Statistical analyses were performed separately for alder and birch litter using *R* version 3.0.1 (*R* Development Core Team 2013). Data were checked for normality and homogeneity of variance following Crawley (2007); response variables were transformed using Box-Cox power transformations when assumptions were not met

(powerTransform function, car package, Fox & Weisberg 2011). Significance was set at $\alpha = 0.05$ for all analyses.

Two-way Analysis of Variance (ANOVA) was used to test the main and interactive effects of CO_2 treatment and microcosm type on each chemical variable (carbon, nitrogen, phosphorus and lignin concentrations, and C/N ratio). Planned contrasts of Least-Square Means (LSM; lsmeans function, lsmeans package, Lenth 2013) were used to compare the effects of CO_2 treatments for each conditioning treatment.

The main and interactive effects of CO_2 treatment and microcosm type were tested on the mass loss of control discs. General Linear Mixed Models (GLMMs) were used to analyse choice control data (lme function, nlme package, Pinheiro *et al.* 2013), where non-independence of discs sharing the same microcosm was accounted for by including microcosm ID as a random term. The same fixed terms were used to analyse control data from the no-choice test using two-way ANOVA.

In the choice test, litter consumption per day was analysed using GLMMs (lme function, nlme package, Pinheiro *et al.* 2013) with the main and interactive effects of CO_2 treatment and invertebrate species as fixed effects and microcosm ID as a random effect. Planned contrasts were performed to compare consumption of ambient- and elevated-CO_2 discs within (i) each invertebrate species, and (ii) invertebrate species grouped by habitat of origin (contrast function, contrast package, Kuhn *et al.* 2011).

In the no-choice test, the main and interactive effects of CO_2 treatment and invertebrate species on litter consumption were tested using two-way ANOVA. Planned contrasts were performed to test the effects of CO_2 treatment on disc consumption within (i) each invertebrate species (lsmeans function, lsmeans package, Lenth 2013) and (ii) invertebrate species grouped by habitat of origin (fit.contrast function, gmodels package, Warnes 2012).

3.4 Results

3.4.1 Litter chemical composition

Carbon dioxide enrichment altered leaf litter chemical composition, but effects differed between tree species. For birch, CO_2-enriched litter contained lower nitrogen concentrations, and higher lignin concentrations and C/N ratios than ambient-CO_2 litter (Tables 3.2 and 3.3). Litter chemical varied between conditioning types, with higher carbon concentrations in aquatically-conditioned litter and lower nitrogen concentrations in terrestrially-conditioned litter (Table 3.2). For both conditioning types, elevated-CO_2 litter contained lower nitrogen concentrations (aquatic, LSM = 0.76% DM, $P < 0.001$; terrestrial, LSM = 1.2% DM, $P < 0.001$; Table 3.3) and higher C/N ratios (aquatic, LSM = 8.3, $P < 0.001$; terrestrial, LSM = 10.3, $P < 0.001$; Table 3.3). For alder litter, the effect of CO_2 treatment was less predictable, with differential responses between conditioning types (Table 3.2). Elevated CO_2 increased alder nitrogen concentrations when conditioned terrestrially (LSM = 0.3% DM, $P = 0.036$; Table 3.3), although there was no concurrent effect in aquatically-conditioned litter (LSM = 0.1% DM, $P = 0.44$; Table 3.3). No treatment or species effects on litter phosphorus concentrations were observed (Tables 3.2 and 3.3).

3.4.2 Invertebrate responses

For both tree species in the choice and no-choice control arenas, disc mass loss in the absence of invertebrates was unaffected by CO_2 treatment and conditioning type ($P > 0.05$). Litter mass loss in the presence of invertebrates was therefore assumed to be a result of invertebrate feeding alone.

In the choice test, leaf palatability affected invertebrate feeding, but this was dependent on tree species. Birch litter consumption was higher for ambient- than elevated-CO_2 discs overall ($F_{1,72} = 10.48$, $P = 0.002$); there was no effect of CO_2 on consumption of alder discs ($F_{1,72} = 187.21$, $P = 0.34$). Consumption also varied between invertebrate species (alder, $F_{7,72} = 0.92$, $P < 0.001$; birch, $F_{7,72} = 30.05$,

Table 3.2. ANOVA summary table of main and interactive effects of CO_2 treatment and conditioning type (CT) on litter chemistry. *P* values < 0.05 are emboldened.

		Carbon		Nitrogen		Phosphorus		Lignin		C/N	
Tree sp.	Variables	$F_{1,16}$	*P*	$F_{1,16}$	*P*	$F_{1,16}$	*P*	$F_{1,12}$	*P*	$F_{1,16}$	*P*
Alder	CO_2	0.6	0.435	1.1	0.305	2.8	0.117	0.04	0.543	1.3	0.271
	CT	0.3	0.577	4.1	0.059	0.2	0.684	0.2	0.673	3.8	0.071
	CO_2 × CT	1.5	0.241	4.7	**0.045**	0.4	0.387	3.6	0.082	4	0.064
Birch	CO_2	0.1	0.712	791	**< 0.001**	3.1	0.098	4.8	**0.048**	605.3	**< 0.001**
	CT	12.1	**0.003**	95	**< 0.001**	0.04	0.848	1	0.331	62.5	**< 0.001**
	CO_2 × CT	3.6	0.077	36.4	**< 0.001**	0.3	0.566	0.1	0.756	6.8	**0.019**

Table 3.3. Chemical composition of leaf litter (expressed as Dry Mass (DM), Dry Cell Wall (DCW), or a ratio; mean ± 1 SEM). Different lowercase letters indicate significant differences ($P < 0.05$) between CO_2 treatments for each tree species × CT combination.

			Elemental composition				Elemental ratios	
Tree species	Conditioning type	CO_2 concentration	Carbon (% DM)	Nitrogen (% DM)	Phosphorus (% DM)	Lignin (% DCW)	C/N	Lignin/N
Alder	Aquatic	Ambient	48.61 ± 0.37a	3.73 ± 0.16a	0.074 ± 0.009a	22.17 ± 2.64a	13.11 ± 0.16a	5.94
		Elevated	48.48 ± 0.25a	3.63 ± 0.091a	0.064 ± 0.009a	19.56 ± 2.74a	13.37 ± 0.36a	5.38
	Terrestrial	Ambient	48.04 ± 0.22a	3.35 ± 0.016a	0.084 ± 0.009a	19.16 ± 1.01a	14.33 ± 0.02a	5.71
		Elevated	48.68 ± 0.40a	3.65 ± 0.026b	0.062 ± 0.01a	24.34 ± 1.14a	13.35 ± 0.10a	6.68
Birch	Aquatic	Ambient	51.22 ± 0.13a	2.54 ± 0.018a	0.09 ± 0.008a	22.10 ± 3.28a	20.17 ± 0.11a	8.7
		Elevated	50.84 ± 0.13a	1.79 ± 0.004b	0.066 ± 0.01a	27.76 ± 1.69a	28.47 ± 0.08b	15.55
	Terrestrial	Ambient	49.86 ± 0.24a	3.08 ± 0.017a	0.082 ± 0.01a	25.09 ± 2.07a	16.19 ± 0.04a	8.15
		Elevated	50.44 ± 0.41a	1.91 ± 0.063b	0.07 ± 0.006a	29.32 ± 1.52a	26.47 ± 0.74b	15.33

$P < 0.001$). The effect of CO_2 on birch consumption varied by invertebrate species ($F_{7,72} = 3.44$, $P = 0.003$), where *O. albicorne* preferred ambient-CO_2 discs (LSM = 1.3 mg d^{-1}, $P < 0.001$; Fig. 3.2b). The effect of CO_2 on litter preference did not vary between invertebrates feeding on alder ($F_{1,72} = 0.5$, $P = 0.83$; Fig. 3.2a). When grouped, aquatic species preferred ambient-CO_2 birch discs over those grown under elevated CO_2 (LSM = 1.1 mg d^{-1}, $P = 0.008$), but no other preferences were exhibited (all $P > 0.05$).

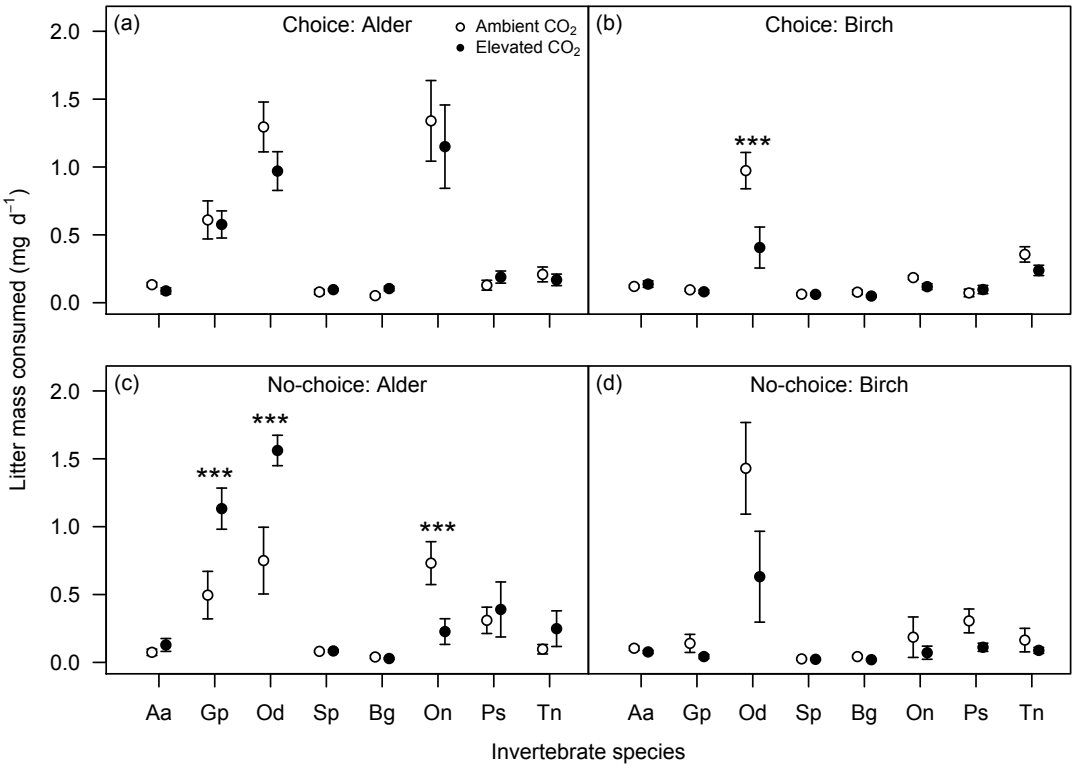

Fig. 3.2. Effects of CO_2 treatment on feeding responses of each invertebrate species. The mean litter consumption (± 1 SEM) of each invertebrate species is shown for (a) alder and (b) birch in the choice test, and (c) alder and (d) birch in the no-choice test. Asterisks indicate significant differences between CO_2 treatments within each invertebrate species (***$P <$ 0.001). Species are arranged by habitat of origin: aquatic species are *Asellus aquaticus* (Aa), *Gammarus pulex* (Gp), *Odontocerum albicorne* (Od) and *Sericostoma personatum* (Sp); terrestrial species are *Blaniulus guttulatus* (Bg), *Oniscus asellus* (On), *Porcellio scaber* (Ps) and *Tachypodoiulus niger* (Tn).

In the no-choice test, consumption rates were higher when invertebrates fed on ambient- rather than elevated-CO_2 birch discs ($F_{1,64} = 6.4$, $P = 0.014$). The trend was

consistent across all invertebrate species, but no individual species showed a significant response (CO_2 treatment × invertebrate species: $F_{7,64} = 0.341$, $P = 0.932$; Fig. 3.2d). This overall effect of CO_2 did not occur in alder leaves ($F_{1,64} = 3.6$, $P = 0.062$), but the effect of CO_2 varied significantly between species ($F_{7,64} = 4.56$, $P < 0.001$); more of the elevated-CO_2 discs were consumed by *G. pulex* (LSM = 2.9 mg, $P = 0.002$) and *O. albicorne* (LSM = 3.2 mg, $P < 0.001$), while *O. asellus* consumed more of the ambient-CO_2 discs (estimate = 2.9 mg, $P = 0.002$; Fig. 3.2c). When grouped by habitat, aquatic invertebrates ate more elevated-CO_2 than ambient-CO_2 alder (LSM = 2 mg, $P < 0.001$) but there was no effect on birch (LSM = 0.1 mg, $P = 0.073$). CO_2 treatment had no effect on consumption by terrestrial species fed either alder (both $P > 0.05$).

3.5 Discussion

Elevated atmospheric CO_2 and microbial conditioning type modified leaf litter chemistry, though effects differed between tree species (supporting Hypothesis 1). Individual invertebrate species varied in their responses, suggesting that caution has to be taken when extrapolating general trends from single-species studies.

Elevated atmospheric CO_2 reduced birch litter quality: the concentration of nitrogen decreased and the C/N ratio increased, regardless of conditioning type. Most species did not respond to this change; *O. albicorne* was the only species with behaviour that supported Hypothesis 2, showing a strong preference for ambient-CO_2 litter. Prior work supports this response: Ferreira *et al*. (2010) showed that low C/N ratios reduced birch litter consumption by the caddis fly *Sericostoma vittatum* Rambur, while Cotrufo *et al*. (1998) found that the woodlouse *P. scaber* preferred high quality (lower C/N ratio and lignin concentration) *Fraxinus excelsior* L. litter grown under ambient CO_2. Alder litter showed negligible chemical change as a result of elevated CO_2, perhaps due to symbiosis with nitrogen-fixing bacteria that help maintain nutrient supplies (Temperton *et al.* 2003). Unexpectedly, a slight increase in quality (increased nitrogen concentration) under elevated CO_2 occurred when alder litter was conditioned terrestrially, but this did not result in any feeding preferences. Effects of conditioning type on litter chemistry may have occurred due to differences in

chemical leaching and microorganism activity between aquatic and terrestrial environments (Treplin & Zimmer 2012). The data indicate that CO_2 enrichment will affect litter palatability to macroinvertebrate detritivores as a result of chemical change, though these effects will be plant and invertebrate species-specific.

In the no-choice test, invertebrates were expected to compensate for low-quality litter by increasing consumption relative to high-quality litter. In contrast to this expectation, compensatory feeding was not observed in either tree species. There was no clear pattern for alder; invertebrate responses were highly idiosyncratic, with *O. asellus* being the only species to consume more of the low-quality resource (terrestrially-conditioned alder litter contained lower nitrogen when grown under ambient CO_2). Hättenschwiler *et al*. (1999) detected a similar compensatory response for *O. asellus* and another woodlouse, *P. scaber*: higher consumption rates were recorded on low-quality, CO_2-enriched *F. sylvatica* litter (low nitrogen concentration, high C/N ratio). The current study showed that *G. pulex* and *O. albicorne* consumed more elevated-CO_2 than ambient-CO_2 alder, despite no observed chemical differences. It is possible that elevated CO_2 reduced litter palatability by altering chemical constituents that were not quantified here, such as secondary metabolites. For example, phenolics and tannins have been shown to be affected by CO_2 levels (Lindroth 2012). Birch litter responses appeared less idiosyncratic, with no individual species increasing consumption of elevated-CO_2 litter. These results suggest that litter species identity determines the predictability of invertebrate feeding responses, but that compensatory feeding is not a unifying trend amongst detritivorous macroinvertebrates.

Feeding rates may have varied due to increased handling times associated with low quality birch litter (e.g. Ott, Rall & Brose 2012), or because of differences in species' body chemistry and their ability to cope with elemental imbalances with CO_2-enriched resources (Martinson *et al.* 2008; Hladyz *et al.* 2009). Heterotrophs, such as the detritivores in the present study, tend to maintain constant body elemental composition (Sterner & Elser 2002) and may alter feeding behaviour to achieve optimum chemical balance. Altered consumption of litter by macroinvertebrates will affect energy release from detritus, in turn affecting secondary production, and food-web structure and functioning (Moore *et al.* 2004). Specifically, on the basis of

invertebrate responses in our study, mineralisation of carbon and nutrients could slow down in forests dominated by birch or other tree species with similar chemistry. This is reinforced by observations of high lignin/N and C/N ratios of elevated-CO_2 birch litter in the current study, which are predictors for slow decomposition rates (Melillo, Aber & Muratore 1982). Conversely, stands containing a lot of alder, or other species with lower C/N ratios, may show little response in terms of detrital processing and nutrient turnover. Differences between tree species make it difficult to predict overall decomposition rates, a task made more difficult by the prevalence of litter mixtures in temperate deciduous forests, which tend to exhibit non-additive decay (Gartner & Cardon 2004).

Changes to litter quality as a result of elevated CO_2 may also affect invertebrate community composition, a potentially important determinant of decomposition rates (Gessner *et al.* 2010). This could be caused by changes to food selection (Hättenschwiler & Bretscher 2001) and increased patchiness of resource quality in litter mixtures on the forest floor (Swan & Palmer 2006b). Differential changes to feeding rates may alter competitive dynamics between invertebrate species, with advantages for species whose dietary breadth extends beyond leaf litter, such *as G. pulex* (MacNeil, Dick & Elwood 1997) and *S. personatum* (Friberg & Jacobsen 1999).

The present study provides, to date, the broadest assessment of detritivorous invertebrate species' feeding responses to CO_2-enriched litter, improving our mechanistic understanding of a key ecosystem process in temperate woodland ecosystems. Future elevations of atmospheric CO_2 are predicted to affect the breakdown of detritus indirectly by reducing leaf litter quality for macroinvertebrate detritivores. The study highlights that this process is highly tree species-specific, and there will be strong responses in some forest stands and minimal effects in others. Identifying the mechanisms governing such ecosystem variation in functional responses to climate change is essential if we are to predict the consequences of elevated CO_2 for forest carbon dynamics and nutrient cycling at regional and landscape-scales.

Author contributions

MWD, TWC, SMT, ADA and THJ conceived and designed the experiments; MWD and SEH performed the experiments; MWD, TWC and SMT analysed the data; DLG, SJO and SEH contributed reagents/materials/analysis tools; MWD wrote the paper; MWD, TWC, SMT, ADA, DLG, SJO, SEH and THJ drafted and revised initial manuscript.

4. Effects of atmospheric change on leaf litter chemical composition and breakdown in a temperate deciduous woodland

4.1 Abstract

Deciduous woodlands are dependent on leaf litter breakdown to drive carbon and nutrient turnover, and to support a diverse community of organisms. This study aimed to understand how this service is threatened by ongoing changes to atmospheric composition, which can alter litter nutritional quality and decay dynamics. *Betula pendula* litter was collected from and compared between (i) ambient CO_2 and elevated CO_2 conditions (produced *ex situ* in a greenhouse), and (ii) rural and urban conditions (collected from *in situ* trees). Litter bags were constructed and exposed to a woodland floor for 0, 28, 56 or 112 days. In terms of chemical composition, ambient- and elevated-CO_2 litters did not differ, but urban litter had lower carbon and nitrogen concentrations than rural litter, along with a higher phosphorus concentration and a higher C/N ratio. In general, litter chemical composition changed after 28 days of exposure, with carbon and nitrogen concentrations increasing, and phosphorus concentration, lignin concentration and C/N ratio decreasing. Regarding decay, there was no difference in the remaining ash-free dry mass of litter between ambient- and elevated-CO_2 litters. Urban litter had consistently less mass remaining at each time period. Litter decay rates (k) were in the order elevated CO_2 > ambient CO_2 > urban > rural. Ambient-CO_2 litter had lower invertebrate richness and diversity than elevated-CO_2 litter, while urban and rural litters did not differ in invertebrate composition. Abundance and richness generally fell through time for all litters, while diversity decreased for ambient- and elevated-CO_2 litters only. Community analysis showed that invertebrate communities differed between time periods. These differences were shaped largely by the relative assemblages of Acari, Chironomidae and collembolan taxa. These results suggest that ongoing atmospheric changes could impact litter chemistry, mass loss and invertebrate community composition, and effects of urban environments may be more important than effects of elevated CO_2.

4.2 Introduction

The majority of primary production in woodlands is fated to enter the detrital pathway, largely as leaf litter (Cyr & Pace 1993; Hairston Jr & Hairston Sr 1993; Cebrian 1999). This material affects the physical and chemical characteristics of woodland floors (Sayer 2006; Xu, Liu & Sayer 2013) and is a key basal resource that influences food web structure (Hagen *et al.* 2012). Detrital decomposition provides a crucial ecosystem function by promoting the release and cycling of carbon and nutrients locked up in organic matter (Moore *et al.* 2004). It is important, therefore, to investigate factors that influence decomposition rates.

Litter nutritional quality is a key driver of litter decay. Breakdown is generally slower for poor-quality litters, which are typified by low nutrient content (e.g. nitrogen and phosphorus) and greater concentrations of recalcitrant carbon-rich compounds, such as lignin (Melillo, Aber & Muratore 1982; Zhang *et al.* 2008; Cornwell *et al.* 2008; Freschet, Aerts & Cornelissen 2012). Changes in chemical composition also occur through the decay process, including the accumulation of nitrogen due to immbolisation by decomposers (McClaugherty, Pastor & Aber 1985; Manzoni *et al.* 2008). Altered chemical composition can affect the activity of detritivorous invertebrates, which are the main organisms responsible for breaking litter into smaller fragments (comminution) by maceration and faecal production (Seastedt 1984; Lavelle *et al.* 2006; Berg & McClaugherty 2008), processes that increase decomposition rates (Wall *et al.* 2008). Invertebrate activity also increases the surface area of litter available for colonisation by saprophagous microorganisms, particularly fungi, which further accelerate decay (Lavelle & Spain 2001; Berg & McClaugherty 2008; Chapin, Matson & Mooney 2011). Changes to leaf litter chemical composition could therefore affect decay rates as mediated by decomposer activity.

Atmospheric concentrations of carbon dioxide (CO_2) and other pollutant gases (e.g. NO_x and SO_x) have been increasing since pre-industrial times (IPCC 2013) Such changes alter the process of litter decomposition in woodland habitats by altering the chemical composition of detritus. Elevated CO_2 generally decreases the nutritional quality of tree leaves by reducing nitrogen concentrations and increasing C/N ratios

(Cotrufo, Ineson & Scott 1998; Coûteaux *et al.* 1999; Gifford, Barrett & Lutze 2000; Lindroth 2010). These chemical changes are maintained after leaves fall as litter, slowing decay on woodland floors (Cotrufo, Ineson & Rowland 1994; Cotrufo, Briones & Ineson 1998; Coûteaux *et al.* 1999; Norby *et al.* 2001). Responses to this material are mixed at the level of litter consumers (Cotrufo, Briones & Ineson 1998; Chapter 3). Urbanised areas have higher concentrations of CO_2 and other pollutants relative to rural areas (Berry & Colls 1990; Ziska, Bunce & Goins 2004; George *et al.* 2007) with further differences in soil chemistry (McDonnell *et al.* 1997). Effects on leaf chemical composition are relatively unknown. Increased deposition of pollutant nitrogen compounds (e.g. NH_4^+, NO_3^-) into the soil may result in greater nitrogen availability to trees (Lovett *et al.* 2000; Zhu & Carreiro 2004; Fang *et al.* 2011), while lignin concentrations may also increase, reducing litter nutritional quality and slowing breakdown rates (Carreiro *et al.* 1999; Pouyat & Carreiro 2003). There is, however, some evidence to suggest that urban litter may decay slower than rural litter (Pavao-Zuckerman & Coleman 2005).

This study compared the effects of atmospheric change on leaf litter chemical composition and the consequences for mass loss and associated invertebrate communities. Experiments were separated to test effects of CO_2 conditions (ambient and elevated CO_2) and urbanisation (rural and urban) on the chemical composition, mass loss, and invertebrate assemblages associated with leaf litter. It was hypothesised that (1) litter chemical composition will differ between atmospheric conditions, with higher quality in (a) urban versus rural litter, and (b) ambient-CO_2 versus elevated-CO_2 litter; (2) litter nutritional quality will increase (i.e. C/N ratio will decrease) through time; (3) litter decay rates will differ between growth conditions, where decay rates of low-quality litter will be slower than for high-quality litter; (4) litters of different quality will support different invertebrate assemblages, with increased invertebrate metrics (abundance, richness and diversity) on higher quality litter; and (5) invertebrate communities will differ between time periods, with a general reduction in invertebrate metrics (abundance, richness and diversity) through time.

4.3 Materials and Methods

4.3.1 Leaf litter production

Leaf litter was collected from silver birch (*Betula pendula* Roth), a widespread deciduous tree species, growing in four different atmospheric conditions: ambient- and elevated-CO_2 litters were produced *ex situ* in a growth facility at Cardiff University, UK, and freshly abscised rural and urban litters were collected *in situ* from mature trees.

One year-old saplings (n = 100; Chew Valley Trees, Bristol, UK) measuring up to 60 cm were planted in pots (height 11 cm and diameter 13 cm) containing John Innes Potting Compost Number 2. Half (n = 50) were grown under ambient CO_2 concentrations (404 ± 1 ppm) and half under elevated CO_2 concentrations (857 ± 8 ppm), with irrigation every two days and ambient lighting for a full photosynthetically-active season (22 March 2012–26 October 2012).

Trees grown under ambient conditions were placed on a bench top within the greenhouse, and temperature and relative humidity were recorded using a digital thermo-hygrometer (Exo-Terra, Yorkshire, UK). Trees grown under elevated CO_2 conditions were distributed equally between ten clear-acrylic closed-top chambers (1.0 × 0.4 × 0.8 m), fed by a closed-loop air delivery system (Fig. 4.1). Fans in an Air Handling Unit (AHU; Diffusion Highline Waterside 260I, size 6) drew air through pre-chamber and post-chamber ducts (200 mm) that split into separate branch pipes (80 mm diameter) for each chamber. Air was monitored every 15 min by two temperature and two CO_2 sensors (Vaisala CARBOCARP Carbon Dioxide Transmitter GM20D), while relative humidity was recorded manually between 0900 and 1100 h using a digital thermo-hygrometer (Exo-Terra, Yorkshire, UK). Automatic responses to temperature and CO_2 sensor readings were controlled by a Building Management System (BMS; TREND IQ 151). When air exceeded pre-set temperatures (> 20°C at 0600–1800 hrs, > 12°C at 1800–0600 hrs), water in the AHU coils was passed to a fan-assisted chilling unit (Daikin air-cooled water chiller EUWY5HB), cooling the air in the system. A solenoid valve controlled the injection

of CO_2 into the system, opening when concentrations fell below 600 ppm and closing when this concentration was exceeded. When opened, CO_2 was introduced to the airflow from a replaceable cylinder (99 kg VK; BOC UK) fitted with a two-stage regulator (200 kPa).

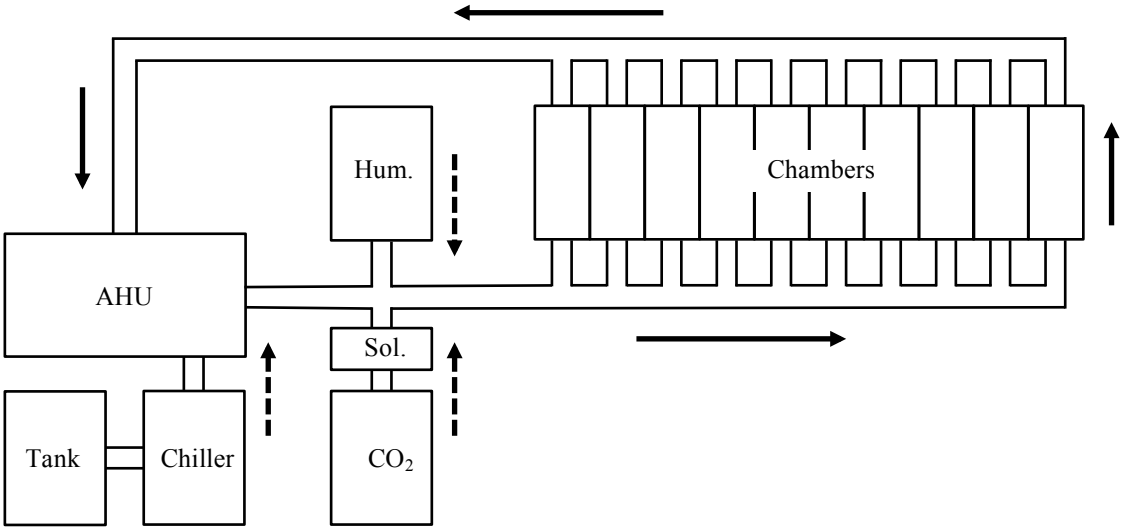

Fig. 4.1. Schematic of the Controlled Environment Facility (AHU = air-handling unit; Hum. = humidifer; Sol. = solenoid valve; CO_2 = carbon dioxide cylinder). Filled arrows show direction of airflow and dashed arrows show inputs to the airstream.

Rural litter was collected from an oak-birch woodland in Ystradffin, Carmarthenshire, UK (52°09'84" N, 3°78'48" W) and urban litter was collected in Grangetown, a residential area of Cardiff, UK (51°47'26" N, 03°18'41" W). Mean values of air pollutants for the five years preceding collection (2006–2010) were taken from recording centres closest to the collection sites. At Aston Hill, Powys (52°50'38" N, 3°03'41" W), mean values of NO, NO_2 and O_3 were 1.2, 7.7 and 65 μg m^{-3}, respectively (Department for Environment Food and Rural Affairs 2013). In Cardiff City Centre (51°48'17" N, 3°17'63" W), values of NO, NO_2, O_3 and SO_2 were 11.7, 31.09, 40.7 and 2.6 μg m^{-3}, respectively (Welsh Air Quality Forum 2013). Litter was air dried immediately on collection and stored separately by growth condition.

4.3.2 Study area

The experiment took place in a temperate deciduous broadleaf forest at Nantrhydifor, Carmarthenshire, UK (52°09'79" N, 03°81'55" W), categorised as a W17b woodland (National Vegetation Classification; Hall, Kirby & Whitbread 2004) dominated by sessile oak *Quercus patraea* (Matt.) Liebl., along with downy birch *B. pubescens* Ehrh. and the fern *Dryopteris dilatata* (Hoffm.) A. Gray. The soil is clayey to silty loam, and acidic.

4.3.3 Litter bags

Litter bags (n = 168) measuring 10 × 15 cm were constructed with 1 mm mesh (EFE & GB Nets, Cornwall, UK) and filled with 3 ± 0.01 g of litter. Bags permitted entry of micro- and mesofauna, which are key litter decomposers (Seastedt 1984; Chapin, Matson & Mooney 2011), and limited losses to non-decay processes (e.g. wind). To assess mass loss and invertebrate assemblages, one bag of each growth condition was attached to a nylon thread that was tied to a labeled 0.5 m steel rod. Nine threads were produced for collection at each of four time periods: 0, 28, 56 and 112 days. Threads allocated to 0 and 28 days were allocated one extra bag of litter from each growth condition, to be used for chemical analyses. Three threads per time period were randomly allocated to each of three blocks placed 20 m apart on a slope gradient. Each block was composed of rods anchored 3 m apart in a randomly-ordered 3 × 3 grid. Bags were placed on the surface of the litter layer. Threads allocated for collection after 0 days were returned immediately to the laboratory for calculation of handling losses and initial litter chemical composition. Bags were placed into separate sealed plastic bags upon collection and returned to the laboratory in a cool box for processing. The experiment ran from 02 November 2012 until 01 February 2013.

4.3.4 Litter chemical composition

Bags allocated for chemical analyses were collected after 0 and 28 days. Litter was washed with deionised water to remove debris (e.g. sediment) and invertebrates,

before being air dried to constant mass and stored at –80°C. Prior to analysis, samples were oven-dried (50°C for 24 hrs) and ground into powder (120 s at 50 beats s^{-1} in a Pulverisette 23 ball mill; Fritsch GmbH, Idar-Oberstein, Germany). Carbon and nitrogen concentrations were determined simultaneously by flash combustion and chromatographic separation of approximately 1.5 mg of ground and homogenised leaf material, calibrated against a standard ($C_{26}H_{26}N_2O_2S$) using an elemental analyser (Elemental Combustion System 4010 CHNS-O Analyzer, Costech Analytical Technologies, Inc., Milan, Italy). Phosphorus was quantified using X-ray fluorescence (see Reidinger, Ramsey & Hartley 2012 for detailed methodology). Carbon, nitrogen and phosphorus concentrations were recorded as a percentage of leaf Dry Mass (% DM). The lignin concentration of litter Dry Cell Walls (% DCW) was determined by following the acetyl bromide spectrophotometric method (Foster, Martin & Pauly 2010). C/N ratios were calculated for each litter sample.

4.3.5 Invertebrate assemblages

Invertebrates were extracted from litter bags using Tullgren funnels (24 hrs) and stored in 70% industrial methylated spirits (Fisher Scientific, UK). Individuals were identified to the lowest practicable taxonomic unit (Acari to order; Annelida to subclass; Collembola to superfamily; Araneae, Coleoptera and Diptera to family; and Diplopoda and Isopoda to species). The following parameters were determined: (i) abundance of each taxon, (ii) richness at the taxon level, and (iii) Simpson's index of diversity, using the equation $1–D = 1–(\Sigma n(n–1)/N(N–1))$, where n is the total number of organisms of a particular taxon and N is the total number of organisms of all taxa.

4.3.6 Mass loss

Following invertebrate extraction, litter samples were washed with deionised water to remove inorganic matter. Litter was air-dried to constant mass (± 0.01 g) and corrected for handling error. This was followed by measurement of Ash-Free Dry Mass (AFDM), where litter was subsampled (0.5 g ± 1 mg) and combusted in a muffle furnace (Carbolite ELF Chamber Furnace 11/14; 550°C for 5 hrs). AFDM was calculated using the equation $AFDM = M_T–[M_T(M_A/M_S)]$, where M_T = dry mass (g) of

the total litter sample, corrected for handling error; M_A = ash subsample mass (g); and M_S = subsample mass (g). The decay coefficient (k) per day was calculated for litter of each growth condition (Petersen & Cummins 1974), using the equation $M_t = M_0(e^{-kt})$, where M_t = AFDM (g) at time t, M_0 = initial AFDM (g), and t = time (days). Values were then used to calculate biological half-life ($t_{1/2}$; time in days to 50% mass loss) using the equation $t_{1/2} = \ln(2)/k$.

4.3.7 Data analysis

Statistical analyses were conducted in *R* version 3.0.2 (*R* Development Core Team 2013), with alpha set at 0.05. For all analyses, separate models were built for (i) litter grown *ex situ* (ambient- and elevated-CO_2), and (ii) litters grown *in situ* (rural and urban), as effects of environmental change were confounded by *in situ* and *ex situ* growing conditions. Models were assessed graphically for normality and homogeneity of variance (Crawley 2007) and were simplified by stepwise removal of non-significant terms ($P > 0.05$) until a minimum adequate model was reached. Significant interactive terms were explored using planned comparisons of Least-Square Means (LSM) between factor levels (lsmeans function, lsmeans package, Lenth 2013)

To assess litter chemical composition, separate General Linear Models (GLMs) were constructed for each chemical variable (carbon, nitrogen, phosphorus and lignin concentrations, and C/N ratio), with the main and interactive effects of growth condition (ambient CO_2 and elevated CO_2, or urban and rural) and time period (0 and 28 days) as explanatory variables.

For litter of each growth condition, dry mass (g) was corrected by adding the mean handling loss of bags collected at Day 0 before calculation of AFDM. Litter mass loss was assessed using a General Linear Mixed-Model (GLMM) with AFDM as the response variable; growth condition (ambient CO_2 and elevated CO_2, or rural and urban), time period (0, 28, 56 and 112 days) and their interaction as fixed categorical explanatory variables; and a random effect term of rod identification number nested within block (lme function, nlme package, Pinheiro *et al.* 2013).

Invertebrate abundance, richness and diversity were analysed using separate GLMMs, with growth condition (ambient CO_2 and elevated CO_2, or rural and urban) time period (28, 56 and 112 days) and their interaction as fixed explanatory variables, and rod identification number nested within block as the random term. Invertebrate abundance was log(abundance+1)-transformed to meet assumptions of normality in the analysis of ambient- and elevated-CO_2 litters.

Differences in invertebrate community composition between growth conditions and time periods were visualised in two dimensions using Non-metric Multi-Dimensional Scaling (NMDS; Kruskal 1964; metaMDS function, vegan package, Oksanen *et al.* 2013). First, a matrix of all pairwise distances was computed using the Bray-Curtis distance measure (4,999 permutations, *adonis* function, vegan package), which is the most suitable measure for zero-skewed data (Clarke & Warwick 2001). NMDS then iteratively assigns samples to a plotting space, attempting to maximise the rank correlation between the plotted distances and pre-calculated distances. Good agreement between these distances lowers 'stress', where a value > 0.3 indicates poor agreement and therefore unreliable graphical interpretability (Zuur, Ieno & Smith 2007). Given the large range in abundances (0–166 individuals), data were fourth-root transformed to down-weight the influence of the most abundant taxa (Clarke & Warwick 2001).

Permutational Analysis of Variance (PERMANOVA; Anderson 2001) is a non-parametric version of multivariate ANOVA that uses permutation techniques to compute *P* values that indicate significant dissimilarities between samples belonging to different groups. An overall PERMANOVA using Bray-Curtis dissimilarities was used to test the response of invertebrate community composition to litter growth condition (ambient CO_2 and elevated CO_2, or rural and urban), time period (28, 56 or 112 days) and their interaction, with iterations constrained within each block (adonis function, vegan package, Oksanen *et al.* 2013). This method is sensitive to unequal variance between treatments, so multivariate homogeneity of group dispersions was assessed (betadisper function, vegan package; Oksanen *et al.* 2013). Factor levels of significant terms in the overall model were compared using pairwise PERMANOVAs. For the analysis of time periods, Bonferroni corrections were used to account for multiple comparisons. Species contributing most to overall community dissimilarity

were identified by Similarity Percentage (SIMPER) analysis (Clarke 1993; simper function, vegan package, Oksanen *et al.* 2013).

4.4 Results

4.4.1 Litter chemical composition

Litter chemical composition differed by growth condition (Fig. 4.2). Urban litter had lower carbon (Fig. 4.2a) and nitrogen (Fig. 4.2b) concentrations, and a higher phosphorus concentration (Fig. 4.2c) than rural litter. The C/N ratio of urban litter was greater than for rural litter, but only for measurements at Day 0 (Fig. 4.2d). Litter chemical composition also changed through time (Table 4.1). For all litters, carbon (Fig. 4.2a) and nitrogen (Fig. 4.2b) concentrations increased, and phosphorus (Fig. 4.2c) concentrations decreased. The lignin concentration fell in ambient-CO_2, rural and urban litters (Fig. 4.2d), and the C/N ratio fell in ambient-CO_2, elevated-CO_2 and urban litters (Fig. 4.2e). Initial lignin/N ratios were 40% higher in ambient CO_2 (27.32) than elevated CO_2 (19.35), and 26% higher in urban (40.91) than rural (32.56) litter. The ratio was reduced after 28 days in the field, narrowing the difference between ambient-CO_2 (14.56) and elevated-CO_2 (15.49) litters, and rural (45.02) and urban (46.62) litters. The mean C/N ratio of ambient- and elevated-CO_2 litters was 46% lower than rural litter, and 63% lower than urban litter. Rural litter had an 8% higher carbon concentration than ambient- and elevated-CO_2 litter, while ambient- and elevated-CO_2 litters had a 39 and 68% higher nitrogen concentration than ambient- and elevated-CO_2 litters, respectively.

4.4.2 Mass loss

Litter AFDM was significantly lower in elevated- than ambient-CO_2 litter ($F_{1,26}$ = 6.94, P = 0.014) and in urban than rural litter ($F_{1,26}$ = 219.7, P < 0.001). AFDM also differed between time periods (ambient and elevated CO_2 litters, $F_{3,26}$ = 769.89, P < 0.001; rural and urban litters, $F_{3,36}$ = 56.16, P < 0.001), decreasing through time

between all pairs of time periods (all $P < 0.001$, except $P < 0.01$ for 28 and 56 days and $P < 0.05$ for 56 and 112 days for *ex situ* litters; Fig. 4.3).

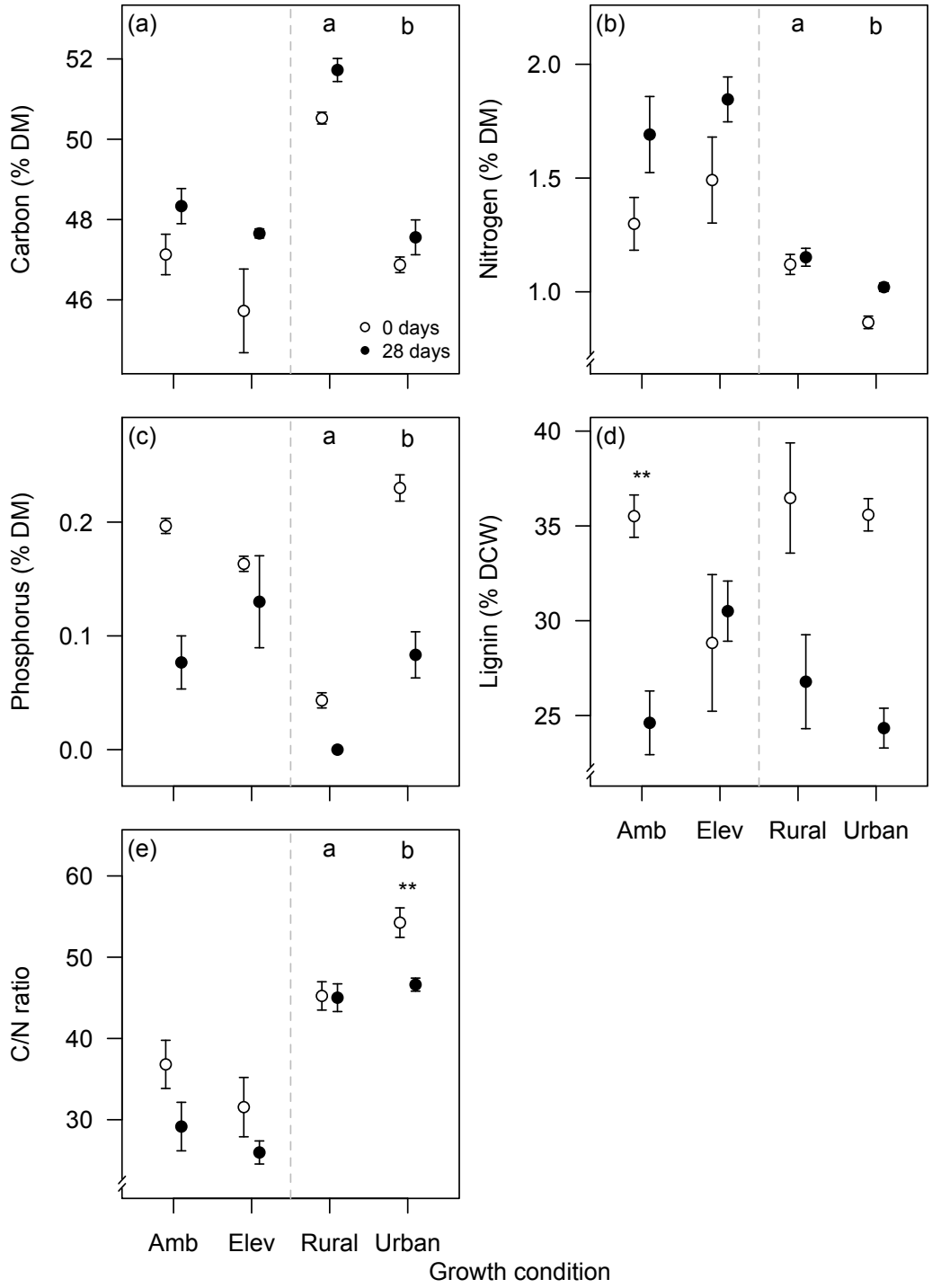

Fig. 4.2. Leaf litter chemical composition (mean ± 1 SEM) following exposure to a woodland floor (DM = Dry Mass, DCW = Dry Cell Wall). Different lowercase letters indicate significant differences ($P < 0.05$) between growth conditions. Asterisks indicate significant differences (**$P < 0.01$, ***$P < 0.001$) between time periods within a growth condition.

Table 4.1. Litter chemical composition in response to growth condition (GC), time period (T), and their interaction (GC × T). Dashes indicate that the parameter was removed during model minimisation. Significant ($P < 0.05$) values are emboldened.

	Carbon		Nitrogen		Phosphorus		Lignin		C/N ratio	
Factor	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*
In situ *(ambient- and elevated-CO_2) litters*										
GC	–	–	–	–	–	–	0.03 (1,8)	0.862	–	–
T	5.7 (1,10)	**0.038**	6.84 (1,10)	**0.003**	9.03 (1,10)	**0.013**	4.35 (1,8)	0.071	5.15 (1,10)	**0.047**
GC × T	–	–	–	–	–	–	118.62 (1,8)	**0.022**	–	–
Ex situ *(rural and urban) litters*										
GC	189.56 (1,9)	**< 0.001**	25.82 (1,9)	**< 0.001**	123.79 (1,8)	**< 0.001**	–	–	11.42 (1,8)	**0.01**
T	10.97 (1,9)	**0.009**	6.02 (1,9)	**0.037**	61.3 (1,8)	**< 0.001**	30.28 (1,10)	**< 0.001**	6.23 (1,8)	**0.037**
GC × T	–	–	–	–	18.13 (1,8)	**0.003**	–	–	5.55 (1,8)	**0.046**

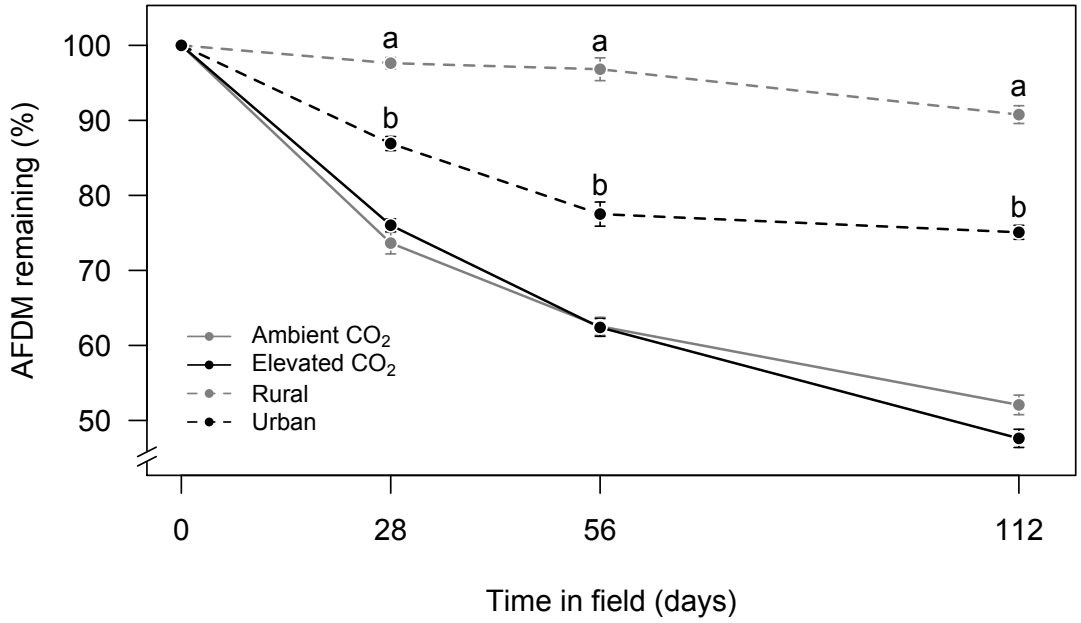

Fig. 4.3. The effect of growth condition on leaf litter Ash-Free Dry Mass remaining (AFDM; mean ± 1 SEM) through time. Lowercase letters indicate significant differences ($P < 0.05$) between growth conditions within each time period.

In the analysis of the *in situ* litters, the effect of growth condition on AFDM differed by each time period ($F_{3,93} = 528.3$, $P < 0.001$), as urban litter had significantly lower AFDM at 28, 56, and 112 days (Fig. 4.3). *Ex situ* CO_2 litters had consistently lower mass than rural (28 days = 34% lower; 56 days = 59%; 112 days = 87%) and urban (28 days = 17% lower; 56 days = 25%; 112 days = 51%) litters through time. Elevated-CO_2 litter had the fastest decay rate and shortest half-life ($k = 0.00663$ day^{-1}, $t_{1/2} = 105$ days), followed by ambient-CO_2 ($k = 0.00582$ day^{-1}, $t_{1/2} = 119$ days), urban ($k = 0.00256$ day^{-1}, $t_{1/2} = 271$ days) and rural ($k = 0.000865$ day^{-1}, $t_{1/2} = 801$ days) litters.

4.4.3 Invertebrate assemblage

Invertebrate abundance differed through time (Table 4.2), increasing from 28 to 56 days for the *ex situ* litters (LSM = 33.2 individuals, $P = 0.048$), and decreasing between 28 and 112 days (LSM = 28.9 individuals, $P < 0.001$), and between 56 and 112 days (LSM = 24.6 individuals, $P = 0.002$), for rural and urban litters (Fig. 4.4a). Invertebrate richness was greater in elevated- than ambient-CO_2 litter (Table 4.2).

Taxon richness also differed between time periods (Table 4.2), decreasing from 56 to 112 days in ambient- and elevated-CO_2 litters (LSM = 1 species, $P = 0.006$), and between 28 and 112 days in rural and urban litters (LSM = 1.8 species, $P = 0.003$; Fig. 4.4b). Invertebrate diversity was higher for elevated-CO_2 litter than ambient-CO_2 litter (Table 4.2), and decreased from 28 to 112 days for these litters (LSM = 0.16, $P = 0.003$; Table 4.2; Fig. 4.4c).

Table 4.2. Response of invertebrate metrics to litter growth condition (GC) and time period (T). Dashes indicate that the parameter was removed during model minimisation; the GC × T interaction was removed from all models. Significant ($P < 0.05$) values are emboldened.

	Abundance		Richness		Diversity	
Factor	*F* (d.f.)	*P*	*F* (d.f.)	*P*	*F* (d.f.)	*P*
In situ *(ambient- and elevated-CO_2) litters*						
GC	–	–	23.64 (1,25)	**< 0.001**	13.11 (1,25)	**0.001**
T	3.5 (2,23)	**0.047**	5.08 (2,23)	**0.015**	5.41 (2,23)	**0.012**
Ex situ *(rural and urban) litters*						
GC	–	–	–	–	–	–
T	9.18 (2,22)	**0.001**	5.31 (2,22)	**0.013**	–	–

Invertebrate community composition was affected by time period for ambient- and elevated-CO_2 litters ($F_{2,48} = 9.19$, $P < 0.001$; Fig. 4.5a), and rural and urban litters, ($F_{2,47} = 6.22$, $P < 0.001$; Fig. 4.5b). Communities differed between 28 and 56 days (rural and urban litters, $t_{1,34} = 3.61$, $P < 0.011$), 28 and 112 days (ambient- and elevated-CO_2 litters, $t_{1,34} = 11.69$, $P < 0.001$; rural and urban litters, $t_{1,33} = 5.98$, $P < 0.001$), and 56 and 112 days (ambient- and elevated-CO_2 litters, $t_{1,34} = 10.43$, $P < 0.001$; rural and urban litters, $t_{1,33} = 8.36$, $P < 0.001$). Results of the analysis on rural and urban litter should be interpreted with caution, as there was evidence for unequal dispersion between time periods in this dataset ($F_{2,50} = 3.36$, $P = 0.043$). The taxa accounting for the largest dissimilarity between time periods were chironomids (ambient- and elevated-CO_2 litters) and collembola taxa (rural and urban litters; Table 4.3).

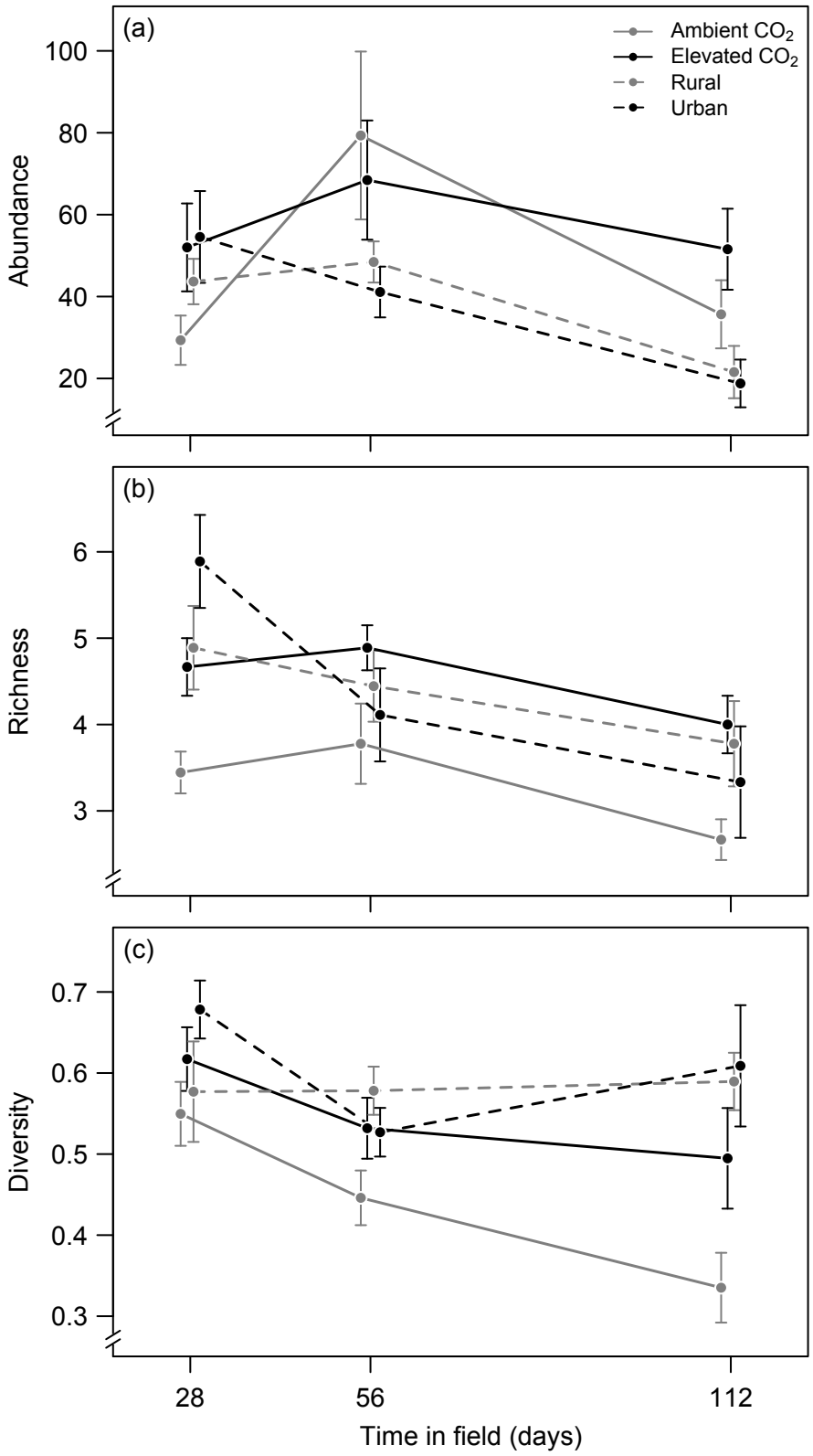

Fig. 4.4. Effects (mean ± 1 SEM) of leaf litter growth condition on (a) abundance, (b) taxonomic richness, and (c) taxonomic diversity (Simpson's index) of invertebrates.

Table 4.3. Taxa accounting for greatest difference between pairs of time periods (days; A vs B) following SIMPER analysis, measured as the percentage contribution of each taxon to the overall dissimilarity between time periods.

Time periods				Abundance (mean ± 1 SEM)	
A	B	Taxon	Contribution	A	B
In situ *(ambient- and elevated-CO_2) litters*					
28	112	1. Chironomidae	23.6%	2.05 ± 0.9	6.22 ± 1.21
		2. Poduroidea	22.8%	8.89 ± 2.34	3.56 ± 1.42
		3. Acari	18%	7.17 ± 0.69	3.61 ± 1.05
56	112	1. Chironomidae	21.3%	5.56 ± 2.9	6.22 ± 1.21
		2. Acari	21%	14.39 ± 2.06	3.56 ± 1.42
		3. Poduroidea	14.1%	4.72 ± 0.84	3.61 ± 1.05
Ex situ (*rural and urban) litters*					
28	56	1. Poduroidea	18.7%	7.67 ± 2.17	2.22 ± 1.16
		2. Chironomidae	14.8%	5.11 ± 1.06	3.06 ± 1.23
		3. Symphypleona	14.7%	3.28 ± 1.13	0.89 ± 0.4
28	112	1. Entomobryoidea	17.3%	17.61 ± 2.42	4.17 ± 1.27
		2. Poduroidea	16.7%	7.67 ± 2.17	1.5 ± 0.87
		3. Acari	14.8%	14 ± 3.24	7 ± 2.3
56	112	1. Entomobryoidea	19.8%	20.17 ± 2.77	4.17 ± 1.27
		2. Acari	18.8%	17.72 ± 2.15	7 ± 2.3
		3. Chironomidae	15%	3.06 ± 1.23	5.83 ± 1.32

4.5 Discussion

The results of this study suggest that ongoing changes to atmospheric gas composition will have variable effects on *B. pendula* litter chemical composition and its subsequent decomposition. There was little difference in the chemical composition of ambient- and elevated-CO_2 litters, and no difference in mass loss, although invertebrate diversity and richness were higher in elevated-CO_2 litter. Conversely, chemical composition differed between urban and rural litters, with urban litter decaying faster but supporting similar invertebrate communities. These results suggest that the storage and cycling of carbon and nutrients in woodland ecosystems could be disrupted by atmospheric change, with implications for food web structure.

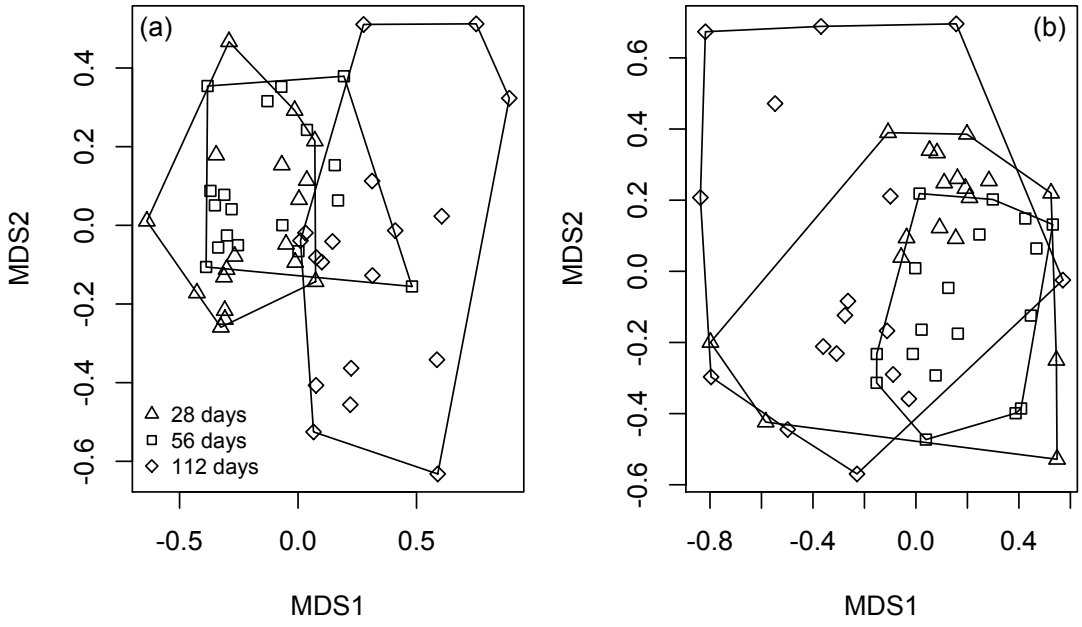

Fig. 4.5. Invertebrate community dissimilarity through time for (a) *ex situ* litters (stress = 0.194), and (b) *in situ* litters (stress = 0.191), visualised using Non-metric Multi-Dimensional Scaling (NMDS).

Litter chemical composition showed some differences between growth conditions. The only difference in chemical composition between ambient- and elevated-CO_2 litters was a higher initial lignin/N ratio in the former, providing poor support for Hypothesis 1a. This was unexpected, as elevated CO_2 tends to reduce nitrogen and increase lignin concentrations of plant litter (Cotrufo, Ineson & Scott 1998; Norby *et al.* 2001). Previous studies of *Betula* species under elevated CO_2 have found changes including increased C/N, lignin/N and phosphorus concentration, along with decreased nitrogen concentration (Parsons, Lindroth & Bockheim 2004; Liu, King & Giardina 2005; Kasurinen *et al.* 2006). Beyond *Betula* species, studies show that changes to chemical composition are species-specific (Coûteaux *et al.* 1999). This includes a lack of response in chemical composition to elevated CO_2, as found for both *Q. cerris* L. and *Q. pubescens* Willd. (Gahrooee 1998). Chemical composition differed between rural and urban litters to a greater extent than for ambient- and elevated-CO_2 litters. In particular, rural litter was of higher initial quality (i.e. lower C/N ratio), providing no support for Hypothesis 1b. This is despite evidence of greater nitrogen deposition into urban soils, potentially allowing for greater uptake of nitrogen into foliar tissues (Lovett *et al.* 2000; Zhu & Carreiro 2004; Fang *et al.* 2011). Urban litter did, however, have a greater phosphorus concentration, but this was not

found in a study of *Quercus rubra* L. leaf tissues grown in urban and rural locations by Baxter *et al*. (2002). Ultimately, the effect of urbanisation on litter quality may be greater than the effects of elevated CO_2, although chemical differences appear to be species-specific and not always predictable based on environmental conditions.

Litter chemical composition changed though time. Quality increased from 0 to 28 days (i.e. C/N ratio decreased) for ambient-CO_2, elevated-CO_2 and urban litters, supporting Hypothesis 2. Increased nitrogen concentration in this study agrees with McClaugherty *et al*. (1985), who also found that nitrogen accumulated in leaf litter samples during nearly two years of breakdown in temperate deciduous forests. This could be due to incorporation of nutrient-rich microbial tissues into the chemical analyses, following colonisation of leaf surfaces by fungi and bacteria (Chapin, Matson & Mooney 2011). Relative reductions of other chemical components, such as phosphorus, may also help explain the relative increase in litter nitrogen concentration. Phosphorus concentrations are, however, species- and site-specific (Gosz, Likens & Bormann 1973; Moore *et al.* 2006). Along with elemental changes, litter structural integrity was reduced in ambient-CO_2, rural and urban litters given reduced lignin concentrations. This is likely due to the release of lignin-degrading enzymes by microorganisms (Berg & McClaugherty 2008).

Differences in litter mass loss between growth conditions were associated with chemical composition, but not in the direction anticipated (that higher leaf litter nutritional quality results in faster breakdown), providing little support for Hypothesis 3. Rural litter was of higher nutritional quality (i.e. lower C/N ratio) than urban litter, but it lost mass more slowly. This is contrary to a study by Carreiro *et al*. (1999), who found that a higher lignin/N ratio and cellulose concentration of urban *Q. rubra* litter resulted in 25% slower mass loss compared to rural litter. This finding also contradicts previous work showing that birch litter decays slower with a higher C/N ratio (Cotrufo, Ineson & Roberts 1995) and that breakdown rate has a positive relationship with nitrogen concentration and a negative relationship with C/N ratio across a range of tree species (Melillo, Aber & Muratore 1982; Taylor, Parkinson & Parsons 1989; Pérez-Harguindeguy *et al.* 2000; Freschet, Aerts & Cornelissen 2012). Urban litter did, however, have a higher phosphorus concentration, which has been linked with faster decay in a global meta-analysis of decomposition (Cornwell *et al.*

2008). Conversely to rural and urban litters, there was little difference in the chemical composition of ambient- and elevated-CO_2 litters, and no difference in mass loss. This contradicts a meta-analysis by Norby *et al*. (2001), showing that elevated atmospheric CO_2 increases the lignin concentration and decreases the nitrogen concentration of leaf litter from woody plants, and slows decomposition relative to ambient-CO_2 litter. Species previously shown to follow this pattern include the current study species *B. pendula* (Cotrufo & Ineson 1996), as well as *F. excelsior*, *A. pseudoplatanus* (Cotrufo, Briones & Ineson 1998) and *Populus* species (Cotrufo, De Angelis & Polle 2005).

Differences in litter chemical composition were not related to differences in community composition, nor were invertebrate abundance, richness and diversity affected, providing no support for Hypothesis 4. Despite this, invertebrate richness and diversity were higher for elevated- than ambient-CO_2 litter, which may reflect lower palatability in the latter due to a higher lignin/N ratio. The study of invertebrate feeding in Chapter 3 showed that four terrestrial invertebrate detritivore species did not show a preference for *A. glutinosa* or *B. pendula* produced under ambient and elevated CO_2, despite a lower nitrogen concentration and higher C/N ratio in elevated-CO_2 *B. pendula*. Compared to the effects of growth condition, there was a greater difference in invertebrate community composition between time periods. This included general reductions in abundance, richness and diversity between 28 to 112 days in the field, supporting Hypothesis 5. These changes may reflect falling substrate availability, where litter at the end of the experiment was composed mostly of tissues with high structural integrity (e.g. midribs) that have low palatability and provide little refuge for invertebrates.

The results of this study imply that elevated atmospheric CO_2 will have little effect on the chemical composition and breakdown of *B. pendula* litter. There was, however, and indication that urban pollution had a greater effect on these parameters. This could affect ecosystem functioning, as detritus provides an important habitat and resource for invertebrate and microbial litter decomposers. At the invertebrate scale, lower litter nutritional quality can reduce palatability (Cotrufo, Briones & Ineson 1998). Differences in invertebrate responses to this low-quality material – as for invertebrate species fed *A. glutinosa* litter in Chapter 3 – could shift invertebrate community structure. In the current study, invertebrate abundance was unaffected by

litter growth conditions, so the availability of invertebrates as a prey item is likely to be unaffected where litter has grown under elevated CO_2 or urban atmospheres. This means that food webs may remain stable, given little impact on feeding by consumers and predators in higher trophic levels (Hagen *et al.* 2012). Invertebrate richness and diversity were higher on elevated- than ambient-CO_2 litter, which could affect decomposition: the number of trophic levels, species identity and the presence of keystone species can all impact litter decay in terrestrial systems (Hättenschwiler, Tiunov & Scheu 2005). Faster breakdown of litter will result in faster release of nutrients and a reduced capacity for carbon storage. Regardless of litter breakdown rates, there could be more detrital inputs to forest floors in the future, as elevated atmospheric CO_2 is expected to increase the amount of leaves produced per plant (Liu *et al.* 2009).

Detritus "often increases system stability and persistence, having substantial effects on trophic structure and biodiversity" (Moore *et al.* 2004). It is therefore of great importance to understand how ecosystems may be affected by changes to detrital chemical composition and breakdown dynamics. This study has shown that changing growth conditions may affect the chemical composition and breakdown of *B. pendula* leaf litter, with a greater relative difference between rural and urban growth conditions than ambient and elevated CO_2 conditions. Further work is required to understand the complex relationship between changing atmospheric composition and decomposition. For example, Leuzinger *et al*. (2011) noted that multiple explanatory variables (e.g. elevated CO_2, warming, drought), longer study duration and larger spatial scales are needed to get a better understanding of the effects of global change on terrestrial systems. It has been argued, however, that climate-related factors – including CO_2 concentration – may not be as important as invertebrate presence and tree species within a system (Gartner & Cardon 2004; Cornwell *et al.* 2008; Rouifed *et al.* 2010). It will therefore be important to explore effects of global change in tandem with multiple species. It is essential that work in this field continues, allowing for a fuller understanding of how ongoing changes to atmospheric composition might affect the crucial ecosystem service of decomposition.

5. Multiple stressor effects on leaf litter chemical composition and breakdown in upland streams

5.1 Abstract

Leaf litter is a major source of nutrients and energy in headwater streams draining temperate woodlands. Litter nutritional quality and decomposition are affected by multiple stressor effects including greenhouse gases, urban pollution and acidification. To identify some of the possible consequences of environmental change, litter bag experiments in acid and circumneutral headwater streams were used to compare chemical composition, decomposition and invertebrate assemblages in *Betula pendula* litter produced (i) under ambient and elevated CO_2 atmospheres *ex situ*, and (ii) in rural and urban locations. Growth conditions affected chemical composition. Elevated CO_2 lowered nutritional quality (nitrogen concentration decreased, and phosphorus concentration and C/N ratio increased), while urban pollution increased it (C/N ratio decreased and nitrogen concentration increased). Once exposed in headwaters, urban litter lost more mass than rural litter through time, while there was no consistent pattern of difference between ambient- and elevated-CO_2 litters. During litter breakdown, environmental stressors had variable effects on invertebrate assemblages. Invertebrate abundance was higher in the circumneutral than the acid stream, but was unaffected by litter source. Taxon diversity was affected by growth condition, but only after 112 days when urban litter held higher invertebrate diversity than rural litter. Invertebrate assemblages differed between streams and between time periods, largely as a result of decreased leuctrid abundances and increased chironomid abundances in later time periods and in acid streams. These results illustrate how atmospheric composition has the potential to alter litter chemical composition and breakdown, but not sufficiently to affect invertebrate use of leaf litter by comparison with acid stress. This could have knock-on effects for nutrient turnover and the stability of food webs in headwater streams.

5.2 Introduction

Ninety percent of forest primary productivity enters the detrital pathway in terrestrial and freshwater ecosystems (Cebrian 1999), largely as leaf litter (Abelho & Graça 1998; Oelbermann & Gordon 2000). Allochthonous litter inputs play a crucial role in trophic structure and nutrient cycling in running waters in particular (Wallace *et al.* 1997, 1999; Moore *et al.* 2004), providing an important energetic resource for invertebrate detritivores ('shredders'; Graça 2001) and fungi (Krauss *et al.* 2011), as well as a substrate for primary producers (e.g. algae; Hax & Golladay 1993). Shredders are among the most important biotic contributors to leaf mass loss (Hieber & Gessner 2002), and by comminution they speed litter decay rates, making recalcitrant nutrients accessible to other organisms (Wallace & Webster 1996). Algal colonisation can increase litter palatability to detritivores (Franken *et al.* 2005) and further stimulate decomposition rates (Rier, Kuehn & Francoeur 2007; Danger *et al.* 2013). While the importance of tree leaf litter to stream ecosystem processes is well established, little is known about the impacts of global environmental change on litter chemical composition and how this will affect the processing and fate of litter in freshwaters.

Fossil fuel combustion has altered both the atmospheric gas concentrations in which plant litter is produced (IPCC 2013) and the chemistry of surface waters in which litter breakdown occurs (Schindler 1988). Atmospheric carbon dioxide (CO_2) concentrations have been particularly affected and are currently 40% higher than in pre-industrial times (IPCC 2013). Elevated CO_2 raises photosynthetic rates in woody tree species, altering growth rates and production (Curtis & Wang 1998; Ainsworth & Long 2005). In turn, foliar chemistry is affected, changing the chemical composition of subsequent litter. At elevated atmospheric CO_2 concentrations, nitrogen concentrations may decrease (Coûteaux *et al.* 1999; Norby *et al.* 2001), while C/N ratios (Cotrufo, Ineson & Rowland 1994; Tuchman *et al.* 2003b), and structural (Norby *et al.* 2001; Tuchman *et al.* 2002; Cotrufo, Drake & Ehleringer 2005) and defensive (Tuchman *et al.* 2003b; Parsons, Lindroth & Bockheim 2004) compounds may increase. Phosphorus concentrations may either increase (Liu, King & Giardina 2007) or decrease (Ferreira *et al.* 2010). Altered leaf chemical composition as a result

of CO_2 enrichment is liable to affect the nutritional quality of litter entering stream food webs (Tuchman *et al.* 2003b). These changes reduce feeding rates by invertebrates (Cotrufo, Briones & Ineson 1998; Ferreira *et al.* 2010), slowing their development and increasing mortality (Tuchman *et al.* 2002, 2003a). In addition, algal colonisation and growth can be affected by litter chemical composition via leachates from decomposing leaves (Friberg & Winterbourn 1996).

Along with the effects of elevated atmospheric CO_2 on litter chemical composition, elevated concentrations of airborne pollutants (e.g. NO^{3-}, NH_4^+) in urban locations further affect litter quality (George *et al.* 2007). Increased soil nitrogen deposition may result in greater nitrogen availability to trees (Lovett *et al.* 2000; Zhu & Carreiro 2004; Fang *et al.* 2011) but outcomes for litter quality (C/N ratio) appear unpredictable (Pavao-Zuckerman & Coleman 2005). Increased concentrations of lignin and labile materials have been identified in urban litter (Carreiro *et al.* 1999) potentially slowing decomposition rates relative to rural litter in forest environments (Carreiro *et al.* 1999; Pouyat & Carreiro 2003).

In surface waters, atmospheric gases from fossil-fuel combustion have also dramatically altered chemical quality. In particular, base-poor soils and waters over large areas of Europe and North America have been acidified by the deposition of strong mineral acidity arising from sulphur and nitrogen oxides which, when dissolved in rainwater, were deposited as 'acid rain' that reduced runoff pH and increased the concentration of metals such as aluminium (Schindler 1988). Although this industrial phenomenon peaked in the 1970s, streams, rivers and lakes have only partially recovered, and are still widely affected by chronic or episodic acidification (Kowalik *et al.* 2007; Ormerod & Durance 2009). The resulting conditions in surface waters may retard leaf litter processing by the combined effects of reduced invertebrate activity (Dangles & Guérold 1998, 2001; Pye, Vaughan & Ormerod 2012) and reduced decomposition by fungi (Krauss *et al.* 2011). The composition of primary producers, particularly diatoms, also change substantially at low pH (Hirst *et al.* 2004), but the effects of changing litter quality and breakdown on these organisms has not been addressed. More significantly, there has been no attempt to identify the combined, multiple-stressor effects of altered atmospheric gas concentrations on litter quality and subsequent breakdown in surface waters affected by acidification.

This study set out to examine the effects of atmospheric CO_2 concentration, urban pollution and stream acidification on leaf litter chemical composition, followed by litter mass loss and invertebrate community metrics and diatom assemblages associated with litter through time. Specifically, it was hypothesised that (1) litter chemical composition will differ between (a) growth conditions, with higher nutritional quality in ambient-CO_2 than elevated-CO_2 litters, and urban litters than rural litters, (b) time periods, with an increase in quality between 0 and 28 days of stream exposure, and (c) streams of differing pH; (2) litter breakdown will differ between (a) litters of different nutritional quality, with faster decay of higher quality litter, and (b) stream of differing pH, with slower decay in the acidified stream; (3) invertebrate communities will differ between (a) litters of different quality, (b) time periods, with a general decline in taxon abundance, richness and diversity through time, and (c) stream pH, with reduced abundance, taxon richness and taxon diversity in acid streams; and (4) biofilm will be more prevalent (a) on leaves with higher lignin concentration (as they are tougher and provide a better substrate), (b) earlier in time sequence, and (c) in the circumneutral than the acidified stream.

5.3 Materials and Methods

5.3.1 Leaf litter growth and production

Leaf litter came from field environments and from artificial rearing facilities (Section 4.3.1) to provide the array of rural, urban and controlled ambient and elevated CO_2 concentrations required for the investigation.

In the controlled facilities, 100 *Betula pendula* Roth (silver birch) trees (Carmarthenshire Tree Nursery, Carmarthen, UK), each one-year old and measuring up to 60 cm, were potted (diameter 13 cm, depth 11 cm; John Innes Potting Compost Number 2) and transferred to a greenhouse (Section 4.3.2). Fifty randomly selected trees were grown in ambient conditions (407 ± 4 ppm) and the remaining 50 in a CO_2-enriched atmosphere (956 ± 16 ppm). Trees were propagated from 16 March–29 October 2011 in ambient light and were watered every 2 days. Leaves of each

treatment were collected upon abscission and stored at room temperature prior to experimentation.

Abscised leaf litters from rural and urban locations were collected in October 2011 from *in situ* silver birch trees. Rural litter was collected from Ystradffin, UK (52°09'75" N, 3°78'91" W) and urban litter from Central London, UK (51°50'81" N, 0°10'01" W). From 1990–2009, the mean daily air temperature at the rural site was 10.4 ± 0.19 ºC and the mean precipitation per month was 57 ± 2 mm; the corresponding readings at the urban site were 10.9 ± 0.22 ºC and 49 ± 2 mm (Microsoft Research 2014). At the nearest air pollution recording sites (Department for Environment Food and Rural Affairs 2013), mean values of atmospheric NO, NO_2 and O_3 in the five full years preceding collection (2006–2010) were 1.2, 7.7 and 65 μg m^{-3} (Aston Hill, Powys; 52°50'38" N, 3°03'41" W), and 20.4, 44.8 and 35.2 μg m^{-3} (Westminster, London; 51°49'46" N, 0°13'19" W), respectively.

5.3.2 *Field study area*

Following litter production and collection, the breakdown experiment was located in two low-order streams within Llyn Brianne Stream Observatory, mid-Wales (52°08' N, 3°45' W), one of the world's longest-running investigations of land use and acid deposition on stream ecosystems (for site details see Durance & Ormerod 2007; Ormerod & Durance 2009). Soft-water runoff (mean total hardness 4–8 mg $CaCO_3$ L^{-1}) occurs at the site as a result of base-poor rocks combining with stagnopodzol, brown podzolic and peat soils. Stream LI1 was acidified (pH 4.9–5.4) as a consequence of interactions between acid deposition and catchment plantations of Sitka spruce (*Picea stichensis* (Bong.) Carrière) and lodgepole pine (*Pinus contorta* Douglas ex Loudon). Stream LI6 was a circumneutral (pH > 6.9) moorland stream buffered by small calcite veins running through its catchment (hardness 15–19 mg $CaCO_3$ L^{-1}).

5.3.3 Litter bags

Litter bags (n = 288) measuring 10 × 15 cm were constructed from 1 mm nylon mesh (EFE & GB Nets, Cornwall, UK), allowing the entry of detritivorous invertebrates while reducing litter loss as a result of physical abrasion. This mesh size was known from previous investigations (Pye, Vaughan & Ormerod 2012) to allow entry of organisms typical of the local shredder community. Each bag was filled with 3 ± 0.01 g (mean ± 1 SEM) of air-dried leaf litter and an embossed plastic identification label and then heat-sealed at the margins. For invertebrate community and mass loss analyses, 240 bags were produced (five time points × two pH levels × four growth conditions × six replicates), along with a further 48 bags for chemical analysis. Bags were randomly assigned to four metal-framed, open-top cages (32.5 × 10.5 × 8.5 cm, 2 × 2 cm minimum aperture) and secured using plastic cable ties. Cages were submerged in a random order along 20 m reaches of each study site and secured with 0.5 m steel rods. Bags allocated to the first time period (0 days) were not placed in-stream, but returned to the laboratory immediately and handling error calculated. The remaining bags were placed in separate sealed plastic bags upon collection (after 14, 28, 56 or 112 days) and transported back to the laboratory in a cool box.

5.3.4 Litter chemical composition

Bags containing litter for chemical analyses were collected after 0 and 28 days. Litter was washed with deionised water, air dried to constant mass and stored at –80°C. Samples were air dried (50°C for 24 hrs) and powdered (120 s at 50 beats s^{-1} in a Pulverisette 23 ball mill; Fritsch GmbH, Idar-Oberstein, Germany) prior to chemical analyses. An elemental analyser (Elemental Combustion System 4010 CHNS-O Analyzer, Costech Analytical Technologies, Inc., Milan, Italy) was used to determine carbon and nitrogen concentrations simultaneously, each expressed as a percentage of leaf dry mass (% DM). This involved flash combustion and chromatographic separation of approximately 1.5 mg of each sample, calibrated against a standard ($C_{26}H_{26}N_2O_2S$). Carbon and nitrogen values were used to calculate C/N ratios for each sample. X-ray fluorescence was used to measure the phosphorus concentration (see

Reidinger, Ramsey & Hartley 2012 for detailed methodology). The acetyl bromide spectrophotometric method (Foster *et al*. 2010) was used to measure the lignin concentration of litter dry cell walls (% DCW). C/N ratios were calculated for each litter sample. Lignin and phosphorus values were derived from separate litter samples to nitrogen values, so lignin/N and N/P ratios were calculated using mean values for each time period × stream pH × growth condition combination.

5.3.5 Invertebrate assemblages

Litter was removed from bags allocated to invertebrate and mass loss analyses, and rinsed in a sieve (500 μm mesh) with deionised water to dislodge invertebrates and inorganic debris (e.g. gravel). Invertebrates were extracted and stored in 70% alcohol before identification to the lowest practicable taxonomic unit (Ephemeroptera and Plecoptera to species; Coleoptera and Trichoptera to genus or species; Diptera to family; Annelida to subclass). The following were calculated for each bag: (i) the total number of individuals (abundance), (ii) the number of taxa (richness) and (iii) Simpson's index of diversity, using the equation $1–D = 1–(\Sigma n(n–1)/N(N–1)$, where n is the total number of organisms of a particular taxon and N is the total number of organisms of all taxa.

5.3.6 Mass loss

After invertebrate removal, the litter was air-dried to constant mass (± 1 mg) and values were corrected for handling error (see below). The ash-free dry mass (AFDM) of litter from each bag was calculated, where subsamples of litter (0.5 g) from each bag were weighed (± 1 mg), before combustion in a muffle furnace (Carbolite ELF Chamber Furnace 11/14; 550°C for 5 hrs). Ash-free dry mass was given by $AFDM = M_T–[M_T(M_A/M_S)]$, where M_T = dry mass (g) of the total litter sample, corrected for handling error, M_A = ash subsample mass (g), and M_S = subsample mass (g).

Breakdown rates per day were calculated using an exponential decay model, following Petersen and Cummins (1974). The decay coefficient, k, was calculated as the slope of the line fitted to each combination of growth condition and stream pH

through time. This was approximated using an exponential decay model in the form $M_t = M_0(e^{-kt})$, where M_t = AFDM (g) at time t; M_0 = AFDM (g) at time 0; k = the decay coefficient; and t = time (days). Values of k were allocated to processing groups as an indicator of breakdown speeds (Petersen & Cummins, 1974): fast ($k < 0.01$), medium ($0.005 < k < 0.01$) and slow ($k < 0.005$). Values of k were used to calculate biological half-life (time to 50% mass loss; $t_{1/2}$), with the equation $t_{1/2} = \ln(2)/k$.

5.3.7 Microalgal biofilm variable chlorophyll fluorescence

A Pulse Amplitude Modulated (PAM) fluorometer (Walz WATER PAM, Heinz Walz GmbH, Germany) with EDF/B fibre optic detector/emitter unit was used to measure diatom activity on leaf surfaces by chlorophyll fluorescence (Maxwell & Johnson 2000). Readings were taken from three leaves per bag. Measurements were taken as soon as possible after removing each bag from the water. Minimum fluorescence (F_0; a proxy for microphytobenthic biomass), and dark-adapted maximum quantum yield of photosystem II (F_v/F_m; an indicator of ecosystem health) were determined from the initial 30 second dark light step of a rapid light curve (Perkins *et al.* 2006). Sigmaplot v14 was used to calculate iterative solutions for each rapid light curve following the method of Eilers and Peeters (1988). This determined the parameters of maximum relative electron transport rate ($rETR_{max}$), light saturation coefficient (E_k) and maximum light use coefficient (α) (for full details see Perkins *et al.* 2006, 2010).

5.3.8 Data analysis

All statistical analyses were performed using *R* version 3.0.2 (*R* Development Core Team 2013) with significance set at $\alpha = 0.05$. Separate models in each analysis were constructed for *ex situ* litters (ambient and elevated CO_2) and *in situ* litters (rural and urban), because effects of CO_2 and urban pollution could not be separated from effects of tree size and age. All models were checked graphically for normality and homogeneity of variance (Crawley 2007). Minimum adequate models were reached by stepwise deletion of non-significant terms. Planned comparisons of factor levels were performed when model terms were significant, using least-square means (LSM; lsmeans function, lsmeans package, Lenth 2013). Three bags allocated to mass loss

and invertebrate analyses – one containing ambient-CO_2 litter and two containing urban litter – were lost from the acid stream at the third time period (56 days) and were excluded from the analyses.

Separate General Linear Mixed-Models (GLMMs) were fitted for each chemical factor (carbon, nitrogen, phosphorus and lignin concentrations and C/N ratio), with growth condition (ambient- or elevated-CO_2, rural, or urban), time period (0 or 28 days), stream pH (circumneutral or acid), and all two- and three-way interactions used as fixed categorical explanatory variables, while cage ID was used as a random term to account for non-independence of litter bags sharing the same cage (lme function, nlme package, Pinheiro *et al.* 2013).

The dry mass (g) of litter from each growth condition was corrected by adding the mean handling loss of bags collected at Day 0 before calculation of AFDM. To compare litter AFDM at each time period, a GLMM (lme function, nlme package, Pinheiro *et al.* 2013) was constructed with AFDM as the response variable and growth condition (ambient CO_2 and elevated CO_2, or rural and urban), stream pH (circumneutral or acid) and days in the field (0, 14, 28, 56 and 112 days) as categorical explanatory variables, and cage ID as a random term.

Separate GLMMs (lme function, nlme package, Pinheiro *et al.* 2013) were constructed for each measure of invertebrate assemblage (abundance, taxon richness and taxon diversity), with growth condition (ambient or elevated CO_2, rural, or urban), time period (14, 28, 56 and 112 days), stream pH (acid or circumneutral), and all interactions as fixed categorical explanatory variables. To account for within-cage variability, cage ID number was included as a random variable. To meet assumptions of normality, invertebrate abundance was log(abundance+1)-transformed in both the analysis of ambient- and elevated-CO_2 litters, and rural and urban litters.

Non-metric Multi-Dimensional Scaling (NMDS; Kruskal 1964) was performed on the invertebrate communities associated with the litter samples (metaMDS function, vegan package, Oksanen *et al.* 2013). Abundances were fourth-root transformed to down-weight the influence of the most abundant taxa (Clarke & Warwick 2001). Bray-Curtis dissimilarity matrices were then constructed with 4,999 permutations

(adonis function, vegan package, Oksanen *et al.* 2013) and the associated stress score was recorded. Permutational Analysis of Variance (PERMANOVA; Anderson 2001) was used to test the effects of growth condition (ambient CO_2, elevated CO_2, rural and urban), stream pH (acid or circumneutral) and time point (14, 28, 56 and 112 days) on invertebrate communities, with iterations constrained within each cage ID (adonis function, vegan package, Oksanen *et al.* 2013). The data were checked for multivariate homogeneity of group dispersions (betadisper function, vegan package, Oksanen *et al.* 2013) before model simplification by stepwise deletion of non-significant terms. For the remaining significant terms, factor levels were compared by pairwise PERMANOVA. Bonferroni-adjusted critical significance levels were used to correct for multiple comparisons. Similarity Percentages (SIMPER; Clarke 1993) analysis was used to determine the invertebrate species that contributed most to the observed dissimilarity between litter samples (simper function, vegan package, Oksanen *et al.* 2013). Further information on these multivariate techniques can be found in Section 4.3.7.

5.4 Results

5.4.1 Litter chemical composition

Growth condition affected the chemical composition of leaf litter, but effects were more pronounced in *ex situ* than *in situ* litters: elevated-CO_2 litter had a lower nitrogen concentration (Fig. 5.1b), higher phosphorus concentration (Fig. 5.1d) and higher C/N ratio (Fig. 5.1c) than ambient-CO_2 litter, while urban litter had a higher nitrogen concentration (Fig. 5.1b) and a lower C/N ratio (Fig. 5.1c) than rural litter (Table 5.1). These differences were present at the start of the experiment and after 28 days of exposure to stream conditions, although the difference in nitrogen concentration between urban and rural litters became more pronounced through time (Fig. 5.1b).

Exposure to stream acidity had a lesser effect on leaf litter chemical composition than growth condition, although *ex situ* litters in the acid stream had greater carbon and

nitrogen concentrations than in the circumneutral stream after 28 days of exposure (Table 5.1). Moreover, stream acidity appeared to interact with the effects of growth condition on *in situ* litter chemical composition (Table 5.1); the nitrogen concentration of urban litter was higher than rural litter, but only in the acid stream (LSM = 0.3 ± 0.1%; $P = 0.004$). The C/N ratio of rural litter was higher in the circumneutral than the acid stream (LSM = 5.4 ± 2.6; $P = 0.036$), but this was not evident in urban litter. The combined effect of these stressors on litter chemical composition was dependent on time period, but only for the *ex situ* litters (Table 5.1): the C/N ratio of elevated-CO_2 litter was higher than for ambient-CO_2 litter, but only after 28 days' exposure to acidified stream conditions (LSM = 3.2 ± 0.8, $P < 0.001$).

Table 5.1. The response of leaf litter chemical composition to growth condition (GC), stream pH (pH) and time period (T), given as *F* value (degrees of freedom), with asterisks indicating significance level ($P < 0.05$*, $P < 0.01$**, $P < 0.001$***; or ns = non-significant). Dashes indicate that a parameter was removed during model minimisation.

Factor	Carbon	Nitrogen	Phosphorus	Lignin	C/N
Ex situ *(ambient- and elevated-CO_2) litters*					
GC	ns	12.61 (1,4)*	20.19 (1,21)***	–	42.09 (1,4)**
T	16.34 (1,12)**	23.45 (1,12)***	126.2 (1,21)***	10.96 (1,14)**	164.9 (1,12)***
pH	8.69 (1,12)*	9.86 (1,12)**	–	–	ns
GC × T	–	–	–	–	ns
GC × pH	–	–	–	–	ns
T × pH	5.59 (1,12)*	–	–	–	ns
GC × T × pH	–	–	–	–	20.07 (1,4)*
In situ *(rural and urban) litters*					
GC	ns	66.25 (1,7)***	ns	–	49.44 (1,8)***
T	ns	38.99 (1,9)***	22.73 (1,11)***	8.18 (1,12)*	24.96 (1,11)***
pH	ns	ns	ns	–	ns
GC × T	ns	11.67 (1,7)*	–	–	–
GC × pH	ns	10.81 (1,7)*	–	–	6.72 (1,8)*
T × pH	ns	–	–	–	–
GC × T × pH	6.27 (1,9)*	–	–	–	–

5.4.2 Mass loss

The effect of growth condition on litter AFDM depended on time period (*ex situ* litters, $F_{4,17} = 3.35$, $P = 0.034$; rural and urban litters, $F_{4,18} = 6.48$, $P = 0.002$): elevated-CO_2 litter had higher AFDM than ambient-CO_2 litter after 14 days (LSM = 0.26 g, $P = 0.006$), but this relationship was reversed after 112 days (LSM = 0.2 g, $P = 0.035$). Conversely, the AFDM of urban litter was lower than for rural litter throughout the manipulation (after 14 days, LSM = 0.47 g; 28 days, LSM = 0.65 g; 56 days, LSM = 0.85g; 112 days, LSM = 0.93 g; all $P < 0.001$; Fig. 5.2). Unlike growth condition, the stressor of stream acidification had no influence on litter AFDM at any time points.

Growth condition had more of an effect on litter decay rates (k) and half-lives ($t_{1/2}$) than stream pH. Elevated-CO_2 litter decayed faster than ambient-CO_2 litter in both the circumneutral (Fig. 5.2a) and acid (Fig. 5.2b) streams, although their half-lives were similar (Table 5.2). Urban litter had a faster rate of decay than rural litter (Table 5.2), but the half-life of rural litter was approximately two-and-a-half times larger in both streams. The *ex situ* litters decayed faster than the *in situ* litters.

Table 5.2. Leaf litter decay characteristics, including the mean Ash-Free Dry Mass (AFDM) at the start and end of the experiment ($n = 3$), the decay constant (k), biological half-life ($t_{1/2}$), and processing groups based on Petersen and Cummins (1974): fast (k (day^{-1}) > 0.01), medium (0.01 > k (day^{-1}) > 0.005), and slow (k (day^{-1}) < 0.005).

Stream pH	Growth condition	Start AFDM (g) ± 1 SEM	End AFDM (g) ± 1 SEM	k (day^{-1})	$t_{1/2}$ (days)	Processing group
Circum-neutral	Elevated CO_2	2.84±0.01	0.28±0.1	0.0206	34	Fast
	Ambient CO_2	2.76±0.02	0.57±0.11	0.0141	49	Fast
	Urban	2.78±0.07	1.21±0.28	0.0074	94	Medium
	Rural	2.91±0.01	2.23±0.14	0.0023	295	Slow
Acid	Elevated CO_2	2.79±0.04	0.42±0.14	0.0170	41	Fast
	Ambient CO_2	2.77±0.02	0.54±0.14	0.0146	48	Fast
	Urban	2.77±0.01	1.3±0.21	0.0068	102	Medium
	Rural	2.89±0.01	2.13±0.21	0.0027	254	Slow

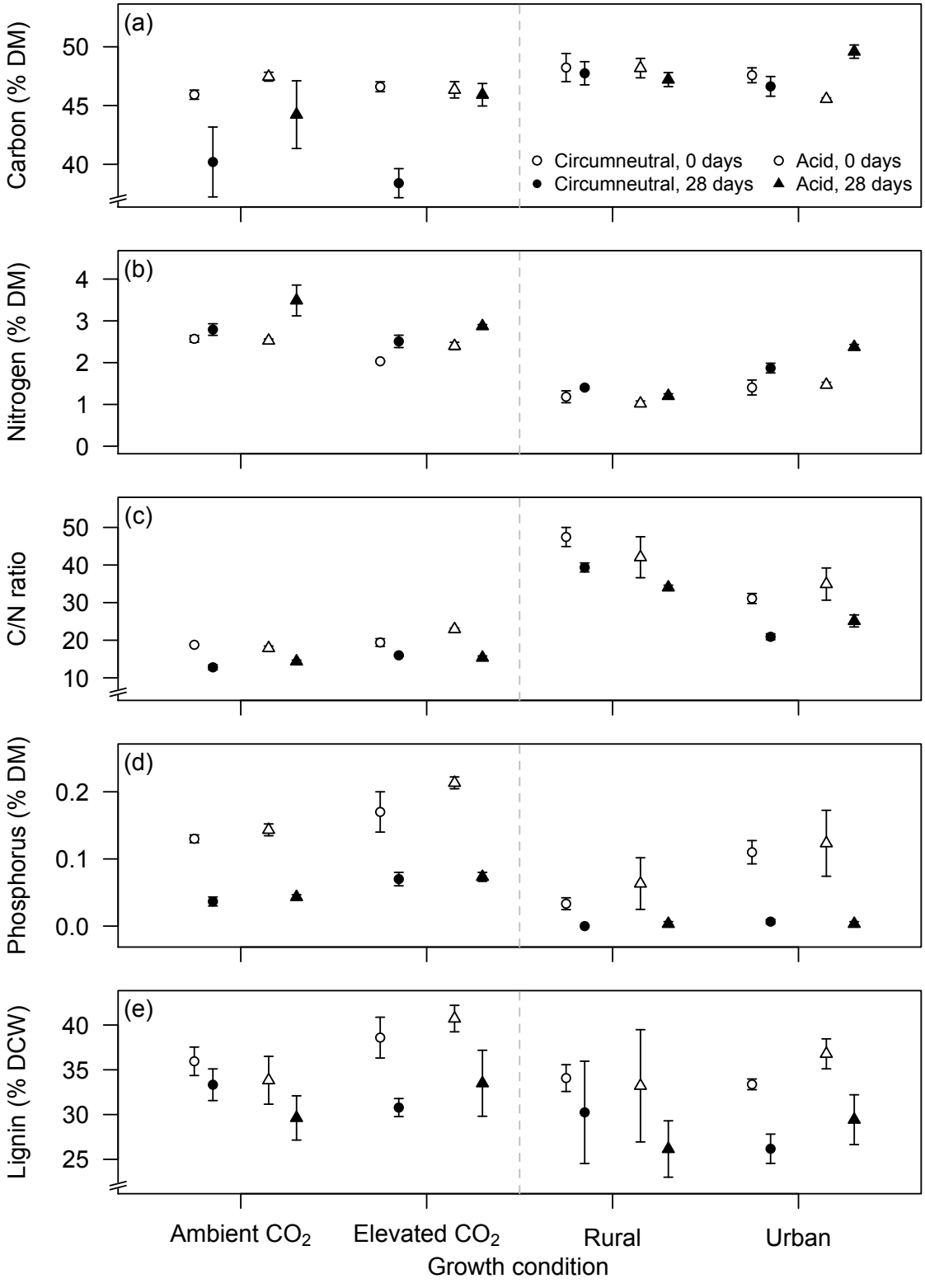

Fig. 5.1. Leaf litter chemical composition (mean ± 1 SEM) through time following exposure to streams of differing pH. Plots show (a) carbon and (b) nitrogen concentrations, (c) C/N ratio, (d) phosphorus and (e) lignin concentrations.

5.4.3 Invertebrate assemblages

In contrast to mass loss, total invertebrate abundance on litter was more affected by stream acidification than litter growth condition: there were more individuals in the circumneutral than the acid stream (*in situ* litters, $F_{1,21} = 5.69$, $P = 0.027$), but there was no effect of elevated CO_2 or urban pollution. Abundance generally fell through time (*ex situ*, $F_{3,25} = 4.76$, $P = 0.009$; *in situ*, $F_{3,21} = 6.31$, $P = 0.003$), being lower after 112 days than after 14 days (*ex situ*, LSM = 1 ± 0.4 individuals, $P = 0.049$; *in situ*, LSM = 1 ± 0.4 individuals, $P = 0.023$), 28 days (*ex situ*, LSM = 1.4 ± 0.4 individuals, $P = 0.002$; *in situ*, LSM = 1.4 ± 0.4 individuals, $P < 0.001$) and 56 days (*in situ*, LSM = 1.3 ± 0.4 individuals, $P = 0.005$) of stream exposure.

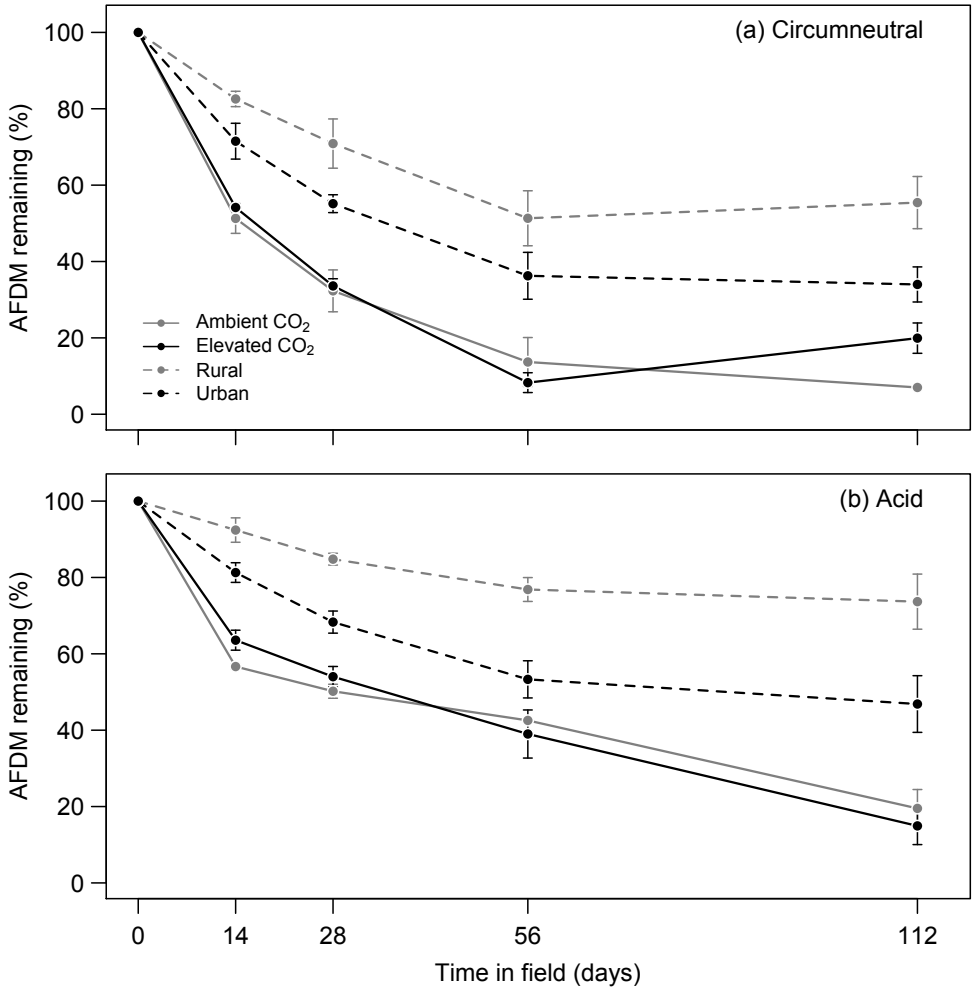

Fig. 5.2. The effect of growth condition on leaf litter Ash-Free Dry Mass remaining (AFDM; mean ± 1 SEM) after exposure to (a) a circumneutral or (b) an acidified stream.

Neither litter growth condition nor stream acidification affected the taxon richness of invertebrates colonising litter. Similarly to invertebrate abundance, taxon richness fell from the start to the end of the experiment (*ex situ*, $F_{3,25} = 4.25$, $P = 0.015$; *in situ*, $F_{3,21} = 6.9$, $P = 0.002$), being lower after 112 days than 14 (*ex situ*, LSM = 2.6 ± 1 taxa, $P = 0.047$; *in situ*, LSM = 0.7 ± 0.2 taxa, $P = 0.007$) and 28 (LSM = 3.5 ± 1 taxa, $P = 0.004$; *in situ*, LSM = 1 ± 0.2 taxa, $P < 0.001$) days of stream exposure. Taxon diversity was unaffected by stream pH, while the effect of growth condition changed through time (*in situ*, $F_{3,14} = 3.69$, $P = 0.038$): diversity was higher on urban than rural litter after 112 days of stream exposure (LSM = 0.4 ± 0.1, $P = 0.006$).

Litter-associated invertebrate communities differed between circumneutral and acid streams for *in situ* litters only ($F_{1,39} = 4.74$, $P < 0.002$; Fig. 5.3c), making stream pH more important than growth condition (Table 5.3): acidification lowered leuctrid abundance, but increased chironomid abundance (Table 5.3). Community composition also varied between time periods for both *ex situ* ($F_{3,27} = 2.03$, $P = 0.015$; Fig. 5.3a) and *in situ* ($F_{3,39} = 4.4$, $P < 0.001$; Fig. 5.3b) litters, differing between 14 and 56 days (*ex situ*, $F_{1,19} = 3.26$, $P = 0.003$), 14 days 112 days (*ex situ*, $F_{1,16} = 4.23$, $P < 0.001$; *in situ*, $F_{1,17} = 7.2$, $P < 0.001$), 28 and 112 days (*in situ*, $F_{1,18} = 5.79$, $P = 0.001$), and 56 and 112 days (*in situ*, $F_{1,16} = 4.09$, $P = 0.008$). Most of these effects were caused by a reduction in leuctrids with progression through the experiment, while chrionomids and oligochaetes increased (Table 5.3).

5.4.4 Algal fluorescence

Fluorescence variables could not be evaluated from most litter bags. As a result, no statistical analyses were undertaken. Patterns of algal activity could not be interpreted (Table 5.4), but the majority of diatom activity was recorded in bags containing rural litter (five out of nine). Algal growth also occurred on the mesh of the litter bags.

5.5 Discussion

Changes in the chemical composition of litter caused by elevated CO_2 and urban pollution could change rates of litter decay, with further impacts on breakdown

processes in acidified streams. The implication is that ongoing changes in atmospheric composition could affect the decay of leaf litter in headwaters, potentially interacting with water quality to impair the provision of an important nutrient source. Such effects have the potential to disturb ecosystem functioning by destabilising river food webs from the bottom-up.

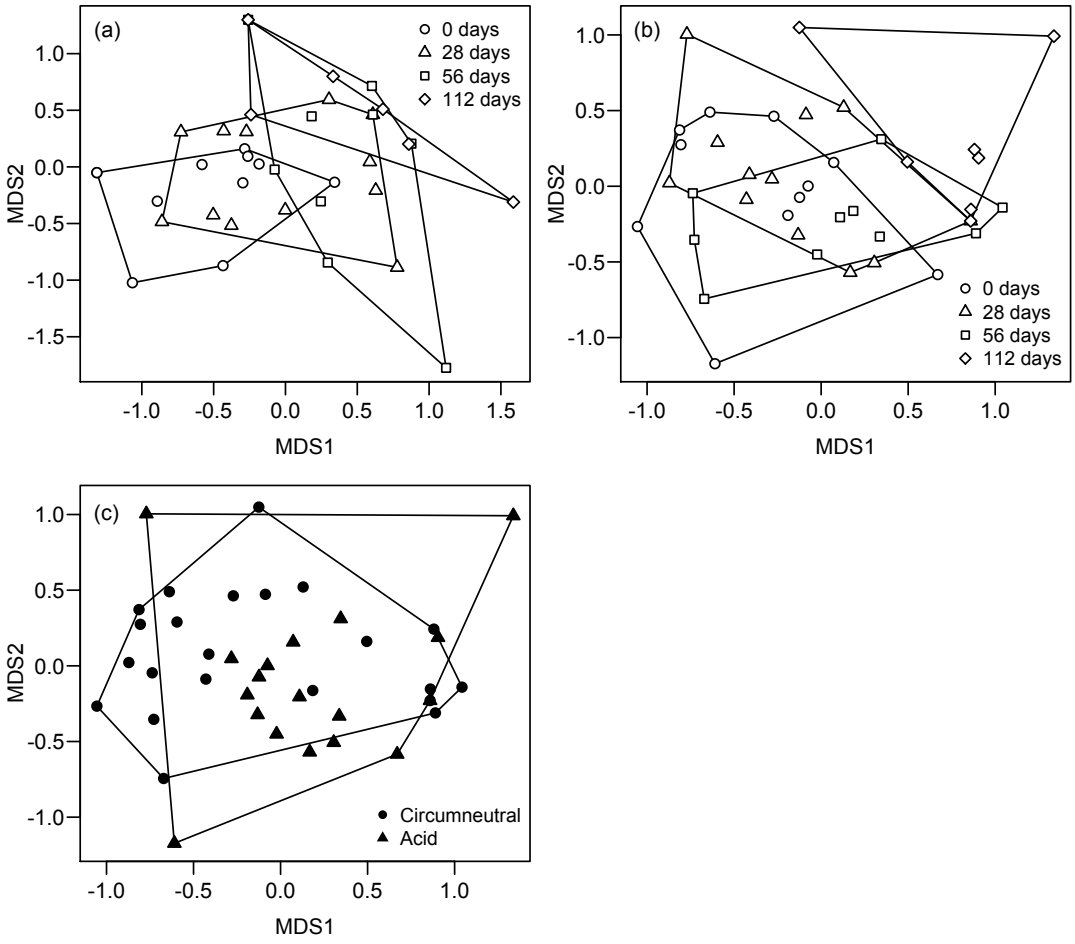

Fig. 5.3. Invertebrate community dissimilarity between time periods for (a) *ex situ* and (b) *in situ* leaf litters, and (c) between streams of differing acidity for *in situ* litters only, visualised using Non-metric Multi-Dimensional Scaling (NMDS; *ex situ* litters, stress = 0.134; *in situ* litters, stress = 0.167).

Litter chemical composition differed between growth conditions, as ambient-CO_2 and urban litters were of higher nutritional quality (i.e. lower C/N ratio) than elevated-CO_2 and rural litters, respectively (supporting Hypothesis 1a). Reduced quality as a result of elevated CO_2 has been observed previously in *B. pendula* (Ferreira *et al.* 2010), with mixed results for other deciduous tree species (Coûteaux *et al.* 1999;

Tuchman *et al.* 2002, 2003b; Rier, Tuchman & Wetzel 2005). One possible explanation is that stomatal number decreases in response to elevated CO_2 (Woodward & Bazzaz 1988), reducing transpiration, and affecting the passage and incorporation of soil-borne nitrogen into the plant (Taub & Wang 2008). Elevated-CO_2 litter had a higher phosphorus concentration than ambient-CO_2 litter, however, which was also true of litter collected from the AspenFACE facility by Liu *et al.* (2007). This may result from greater carbon availability to exchange for soil-derived phosphorus with mycorrhizae, standing stocks of which have been shown to increase under elevated CO_2 (Treseder 2004). Urban litter may have been of higher nutritional quality than rural litter as a result of greater nitrogen deposition (Lovett *et al.* 2000; Zhu & Carreiro 2004; Fang *et al.* 2011) and phosphorus availability (Zhang & Ke 2004) in urban soils. Litter quality also appeared to be affected by artificial growth conditions, as greenhouse-grown (ambient and elevated CO_2) litters were of higher initial quality than outdoor-grown (urban and rural) litters. This is likely due to optimal growth conditions under greenhouse conditions (e.g. optimal soil nutrients, temperature or irrigation), or that sapling litter was of higher quality than that of mature trees. This has important ramifications for the interpretation of experiments using leaves or litter produced under artificial conditions.

Litter chemical composition changed after 28 days of stream exposure, including increased quality of all litters (i.e. C/N ratio decreased), supporting Hypothesis 1b. The reduction in phosphorus concentration is likely to reflect its high solubility, resulting in rapid loss from leaf litter during the leaching phase (Abelho 2001). Increased nitrogen concentration has been observed previously for *Alnus glutinosa* (L.) Gaertn., *Castanea sativa* Mill. and *Quercus faginea* Lam. decomposing in a low-order Portuguese stream (Canhoto & Graça 1996). One possibility is that this reflects increased fungal biomass during the conditioning phase (Abelho 2001; Krauss *et al.* 2011), resulting in incorporation of fungal tissues into the chemical analyses. Incorporation of nitrogen from fungal biomass could be expected to be lower in acidified streams, given evidence of fungal preference for circumneutral streams (Hall *et al.* 1980; Griffith & Perry 1994), but stream pH had little effect on litter chemical composition, providing no support for Hypothesis 1c.

Litter mass loss appeared to be linked to litter nutritional quality: urban litter was of higher quality than rural litter, and had significantly lower mass at every time point (supporting Hypothesis 2a). Conversely, there was little to separate mass loss between ambient- and elevated-CO_2 litters, despite the higher quality of ambient-CO_2 material. Despite this, ambient-CO_2 litter lost significantly more mass than elevated-CO_2 litter after 14 days.

Table 5.3. Litter-associated taxa accounting for the greatest difference between pairs of time periods (days) and stream pH following SIMPER analysis, measured as the percentage contribution (%) of each taxon to the overall dissimilarity between contrasts (A vs B).

Contrast				Abundance (mean ± 1 SEM)	
A	B	Taxon	%	A	B
Ex situ *(ambient- and elevated-CO_2) litters*					
14	56	1. *Leuctra inermis*	18	2.33 ± 0.8	0.55 ± 0.55
		2. Chironomidae	15	0.92 ± 0.4	1.55 ± 0.55
		3. *L. hippopus*	14	2.17 ± 0.99	0.09 ± 0.09
14	112	1. *L. inermis*	19	2.33 ± 0.8	0.08 ± 0.08
		2. Chironomidae	16	0.92 ± 0.4	0.92 ± 0.5
		3. *L. hippopus*	15	2.17 ± 0.99	0
In situ *(rural and urban) litters*					
14	112	1. *L. inermis*	16	3.17 ± 0.83	0.08 ± 0.08
		2. Chironomidae	14	0.67 ± 0.22	1.75 ± 0.78
		3. Oligochaeta	14	0	0.83 ± 0.37
28	112	1. *L. hippopus*	13	3.08 ± 1.25	0.25 ± 0.25
		2. Oligochaeta	12	0.83 ± 0.83	0.83 ± 0.37
		3. Chironomidae	10	1.67 ± 0.41	1.75 ± 0.78
56	112	1. Oligochaeta	17	0.1 ± 0.1	0.83 ± 0.37
		2. *L. inermis*	16	3.1 ± 1.4	0.08 ± 0.08
		3. Chironomidae	16	3.6 ± 1.44	1.75 ± 0.78
Circumneutral	Acid	1. *L. inermis*	14	4 ± 0.97	1.18 ± 0.54
		2. Chironomidae	12	1.38 ± 0.42	2.36 ± 0.72
		3. *L. hippopus*	11	2.21 ± 0.83	0.86 ± 0.42

This is similar to the findings of Rier *et al*. (2002) and Tuchman *et al*. (2003b). These studies showed that *Populus tremuloides* Michx. decay rates were slower for elevated-CO_2 litter after 30 days of stream exposure, with no differences after 60, 90 and 120 days. This suggests that the effects of initial chemical quality on litter decay may occur over the early stages of decay in some cases.

Table 5.4. Algal fluorescence parameters ($rETR_{max}$, maximum relative electron transport rate; α, maximum light use coefficient; E_k, light saturation coefficient) recorded from leaf litter and litter bag surfaces.

Days	Source	Stream pH	Growth condition	$rETR_{max}$	α	E_k
14	Litter	Acid	Elevated CO_2	54.42	0.279	224.84
			Rural	66.78	0.3	224.84
	Litter	Circumneutral	Rural	44.92	0.21	214.39
	Bag	Circumneutral	Rural	39.44	0.265	148.7
28	Litter	Acid	Ambient CO_2	34.06	0.016	92.56
	Litter	Circumneutral	Rural	28.1	0.166	169.55
56	Litter	Acid	Rural	43.37	0.273	158.62
			Urban	25.06	0.215	116.67
	Litter	Circumneutral	Rural	70.31	0.253	278.32
			Urban	61.6	0.251	278.32
	Bag	Circumneutral	Elevated CO_2	44.28	0.38	116.55

Along with litter quality, leaf mass was affected by stream pH, with lower AFDM in the circumneutral stream (supporting Hypothesis 2b), though the effect was small and only occurred for ambient- and elevated-CO_2 litters. This supports prior work showing reduced mass loss in acidified streams (Griffith & Perry 1994; Merrix, Lewis & Ormerod 2006), including at Llyn Brianne (Pye, Vaughan & Ormerod 2012), but the effect was much weaker than in studies such as Dangles *et al*. (2004), which showed that breakdown was over 20 times slower under acid conditions.

Decay proceeded at a comparable rate to other *Betula* species, although there are few studies of this genus and large variation between available data. For example, the decay rate (*k*) of *B. pubescens* ranged from 0.0085 to 0.0331 day^{-1} across several Scottish streams (Collen, Keay & Morrison 2004); *B. lenta* decayed at 0.004 to 0.01

day^{-1} in North Carolina, USA (Meyer & Johnson 1983); and *B. pubescens* at 0.0033 day^{-1} in Central Spain (Escudero *et al.* 1991). The clearest discrepancy in the current study was between greenhouse-produced (ambient and elevated CO_2) litters and outdoor-grown (rural and urban) litters. The former was categorised as 'fast' decay according to Petersen and Cummins (1974), and the latter as 'medium' or 'slow'. This appears to be linked to litter chemical composition, as high quality ambient- and elevated-CO_2 litters broke down faster than lower-quality urban litter, which in turn broke down faster than the lowest quality litter from the rural growth condition. In general, decay coefficients were typical of deciduous litter (Abelho 2001).

Invertebrate abundance and richness generally decreased from the early to late stages of the experiment, while community dissimilarity tended to be greatest between the early and latter stages of the experiment (supporting Hypothesis 3b). This is likely to be a result of reduced substrate availability: litters with greater AFDM tended to support a higher abundance and richness of invertebrates. The switch from coarse to fine particulate organic matter within the bags was reflected in a switch from shredding species (e.g. *Leuctra* species) to those that consume detritus that has become more sediment-like (e.g. Oligochaeta). There was little effect of growth condition on any measure of invertebrate assemblages, providing poor support for Hypothesis 3a. This is surprising, given that shredders are sensitive to litter quality (Irons, Oswood & Bryant 1988; Graça, Cressa & Gessner 2001; Tuchman *et al.* 2002, 2003a) and might be expected to have greater abundance on higher quality litters in this experiment.

For rural and urban litters, invertebrate communities were more affected by stream acidity than litter growth condition. This agrees with prior work showing that acidification causes impoverished invertebrate communities (Mackay & Kersey 1985; Simpson, Bode & Colquhoun 1985; Sutcliffe & Hildrew 1989), supporting Hypothesis 3c. This reinforces prior findings from Llyn Brianne showing that, despite some recovery, acidified streams still deter sensitive species (Ormerod & Durance 2009). Shredders appeared to be particularly affected by acidity. For example, acid-sensitive chironomids (Orendt 1999), and the putative shredders *L. inermis* Kempny and *L. hippopus* Kempny were less abundant in the acidified stream. Shredder reduction has been observed in acidified streams before (Dangles 2002), but not in

every instance (Dangles *et al.* 2004). One mechanism for reduced shredder abundance is that stream acidity reduces fungal biomass on litter (Griffith & Perry 1994), lowering palatability to invertebrates (Bärlocher 1985; Graça, Cressa & Gessner 2001). This may, in turn, explain the reduced decay rate of litter observed in the acidified stream, a pattern found in the breakdown of other deciduous species (Griffith & Perry 1994; Dangles *et al.* 2004; Merrix, Lewis & Ormerod 2006). Invertebrates may also be physically intolerant of acidified conditions, while reduced litter availability in acidified streams may also be responsible for impoverished communities (Rosemond *et al.* 1992).

Little biofilm activity was recorded on leaf material in any combination of time period, growth condition and stream pH; there was therefore no evidence to support Hypothesis 4. Light penetration may have been limited by the mesh material and by tight packing of litter, limiting photosynthetic activity within the bags. While the potential for algal colonisation of leaf litter was established, no effect of algal-assisted decomposition could be observed. The effect of biofilm development on leaf litter has been shown to influence breakdown rates (Rier, Kuehn & Francoeur 2007; Danger *et al.* 2013), but the question of how multiple stressors affect algal colonisation of leaf litter – and its subsequent decomposition – remains unresolved.

This study further confirms that atmospheric growth conditions can affect litter quality and breakdown, and that acidity remains a persistent problem for ecosystem functioning in headwater streams. Detritus is an important component of most ecosystems (Moore *et al.* 2004) and is particularly important in stream habitats (Wallace *et al.* 1999). Changes to mass loss as a result of altered chemistry and exposure to acid stream conditions could affect standing stocks of litter, which are an important carbon store (Meyer, Wallace & Eggert 1998). Leaching rates appear to be correlated with nutrient concentrations (Gosz, Likens & Bormann 1973), so litter chemistry change, as a result of altered growth conditions, could result in changes to the release and transport of nutrients downstream. This could disrupt food web structure and functioning, change invertebrate trophic composition (Wallace *et al.* 1997), and alter food availability to top predators, such as fish (Wallace & Webster 1996) and birds (Steinmetz, Kohler & Soluk 2003).

The experimental results indicated that litter chemical composition was affected by growth condition, but this did not necessarily result in major differences in mass loss or invertebrate assemblages, nor was there a consistent effect of stream pH across the litter types. This variability highlights the need for further work to understand better how tree litter decay will respond to ongoing environmental changes. For example, future studies could involve the use of stressor gradients and additional tree species to help elucidate general mechanisms and to predict the response of litter decay to the interactive effect of atmospheric change and stream acidification. It is, however, clear that changes to litter chemical composition and stream acidity are important factors to consider when evaluating the future of freshwater functioning, particularly with respect to decomposition and associated faunal activity.

6. Effects of elevated CO_2 on twig chemical composition and subsequent decay in terrestrial and acidified aquatic environments

6.1 Abstract

Small woody debris (SWD) is an important but overlooked resource in temperate deciduous woodlands and adjacent streams. Its breakdown results in the gradual release of stored carbon and nutrients to the environment, helping to support food webs and nutrient cycling. Global change processes threaten this function. For example, the decay of SWD is related to its chemical composition, but little is known about how this linkage might be affected by ongoing increases in atmospheric CO_2 and stream acidification. To investigate these effects, twigs of *Betula pendula* were produced under ambient and elevated CO_2, before exposure to a woodland floor or forested headwater streams of acidic and circumneutral pH. Regardless of habitat, initial lignin concentrations were higher in elevated-CO_2 twigs, implying lower nutritional quality, while carbon concentrations also increased through time. In the aquatic study, nitrogen concentration increased through time in the circumneutral stream, but not the acidified stream, while the C/N ratio decreased through time. The proportion of twig mass remaining at the end of each experiment was lower for elevated-CO_2 twigs in both the aquatic and the terrestrial environments, despite the perceived lower quality of this material. Breakdown rates differed between habitats, as exponential decay constants were lower in the terrestrial (k = 0.05–0.091 year^{-1}) than the aquatic (k = 0.216–0.277 year^{-1}) experiment, which may result from greater physical abrasion in the stream environment. These results indicate that the stressors of elevated CO_2 and stream pH can affect nutrient and breakdown dynamics of SWD. This could cause increased retention of SWD in these environments, enhancing its role as a carbon and nutrient store, but resulting in slowed release of these resources to terrestrial and aquatic organisms.

6.2 Introduction

The majority of primary productivity in temperate deciduous forests is allocated to wood production (Luyssaert *et al.* 2007), and approximately one quarter of this material is dead at any given moment (Thomas & Packham 2007). Studies of dead wood have tended to focus on large woody debris (e.g. logs and branches) rather than Small Woody Debris (SWD), which is generally defined as sticks and twigs with a diameter of 10 cm or less (Harmon *et al.* 1986; Kirby *et al.* 1998; Dearden *et al.* 2006; Thomas & Packham 2007). Despite this, SWD can be ubiquitous (Harmon *et al.* 1986; Dearden *et al.* 2006): for example, approximately 20% of the litter generated in temperate deciduous woodlands is SWD (Gosz, Likens & Bormann 1972), and it composes approximately 60% of the coarse matter standing crop of adjacent streams (Abelho & Graça 1998). Studies of SWD decomposition dynamics have largely been restricted to commercially modified substitutes, such as tongue depressors (Arroita *et al.* 2012), veneers (Hofer & Richardson 2007) or chips (Melillo *et al.* 1983), which can have different sizes, shapes, and area-to-volume relationships compared to natural material (Spänhoff & Gessner 2004). More work is required therefore to unravel the breakdown of natural SWD in woodland ecosystems.

Woody litter is an important resource in woodlands, delivering a range of services to both terrestrial and aquatic environments. For example, it provides habitat for primary producers and invertebrates, such as mosses, algae, woodlice and caddis flies (Harmon *et al.* 1986; Eggert & Wallace 2007; Hofer & Richardson 2007). Microbes and xylophagous invertebrates can also take advantage of woody debris as a nutrient source (Anderson *et al.* 1978; Tedersoo *et al.* 2003; Berg & McClaugherty 2008). Its sporadic appearance in time and space (Kirby *et al.* 1998; Berg & McClaugherty 2008) makes woody litter a useful supplementary nutrient source for decomposers outside of peak leaf-litter fall in autumn (Gosz, Likens & Bormann 1972; Abelho & Graça 1998). Furthermore, woody 'jams' can promote pool formation in streams, contributing to habitat heterogeneity and limiting losses of organic matter downstream (Bilby & Likens 1980; Bilby 1981). Given its importance to woodland ecosystems, it is crucial to understand how SWD's role as a resource and habitat

modifier might be affected by environmental changes and subsequent alterations to woody traits.

The chemical composition of woody litter influences its breakdown. For example, a global meta-analysis of angiosperm wood decay by Weedon *et al*. (2009) found that higher nitrogen concentrations and lower C/N ratios correlate with faster decay. Ongoing changes to atmospheric gases can influence litter chemistry and could therefore alter breakdown. Carbon dioxide (CO_2) is of particular note, as it has been steadily increasing in concentration following the advent of industrialisation (IPCC 2013). The effect of elevated CO_2 on woody litter chemical composition is unclear, however, having been shown to increase (El Kohen, Rouhier & Mousseau 1992), decrease (Cotrufo & Ineson 2000) or have no effect (Williams *et al.* 1986) on nitrogen concentrations. Similarly, elevated CO_2 can either increase (Richet *et al.* 2012) or decrease (Cotrufo & Ineson 2000) lignin concentrations. Furthermore, chemical changes as a result of elevated CO_2 do not necessarily result in changes to mass loss and nutrient dynamics through time (Cotrufo & Ineson 2000). Further work is required to link atmospheric composition with litter chemical composition and mass loss, which will help untangle the relationship between global change and the essential ecosystem process of litter decomposition.

Freshwater acidification is another stressor frequently linked with global change that could affect wood decay. Pollutants dissolved in rainwater – particularly sulphur and nitrogen oxides – have reduced runoff pH, acidifying headwater streams in Europe and North America. Chronic or episodic acidity is still a threat to stream habitats despite some signs of recovery (Kowalik *et al.* 2007; Ormerod & Durance 2009). Acidity can affect the breakdown of organic material: leaf litter was shown to decompose up to 20 times slower in acid than in circumneutral streams (Dangles *et al.* 2004), an effect partially due to reduced decomposer activity (Dangles & Guérold 1998, 2001; Krauss *et al.* 2011; Pye, Vaughan & Ormerod 2012). It is important to understand how acidification might affect the process of woody decomposition and whether the effects are altered by the changing composition of the atmosphere.

The aim of this study was to compare the chemical composition and breakdown of woody debris – produced under ambient and elevated CO_2 – on a woodland floor, and

in headwater streams of contrasting pH. The following hypotheses were tested: (1) elevated CO_2 will alter twig chemical composition, resulting in lower quality compared to ambient-CO_2 twigs (i.e. the C/N ratio and lignin concentration will be reduced), (2) the proportion of twig mass remaining at the end of each experiment will be lower for ambient-CO_2 twigs as a result of higher quality, and (3) twigs exposed to acid streams will decompose more slowly than in circumneutral streams.

6.3 Materials and Methods

6.3.1 Twig litter production

The trees used in this study were the same as those used for the studies reported in Chapters 4 and 5. Two batches of 100 *Betula pendula* Roth (silver birch) trees were grown over separate seven-month growing periods (March–October 2011 and 2012) in a growth facility at Cardiff University, UK (see Section 4.3.2 for details). Trees were all a year-old and measured up to 60 cm in height (2011, Carmarthenshire Tree Nursery, Carmarthen, UK; 2012, Chew Valley Trees, Bristol, UK), and were potted (diameter 13 cm, depth 11 cm) in John Innes Potting Compost Number 2. Half of the saplings were produced under ambient CO_2 concentrations (2011, 407 ± 4 ppm; 2012, 404 ± 1 ppm) and half under elevated CO_2 concentrations (2011, 956 ± 16 ppm; 2012, 857 ± 8 ppm). At the end of the growing season, aboveground woody material was harvested, cut into 'twigs' (10 cm long; 3–6 mm diameter), oven-dried (50°C for 48 hrs), and stored prior to experimental use. Twigs produced in 2011 were used in the aquatic study and those from 2012 were used in the terrestrial study.

6.3.2 Study area

Terrestrial decomposition of twigs took place at Nantrhydifor, Carmarthenshire, UK (52°05'52" N, 3°48'57" W), a temperate deciduous broadleaf forest classed as W17b woodland (National Vegetation Classification; Hall *et al*. 2004). Sessile oak *Quercus ,patraea* (Matt.) Liebl., is the dominant species, with intermittent downy birch, *Betula pubescens* Ehrh., and the fern *Dryopteris dilatata* (Hoffm.) A. Gray. The

underlying bedrock is Silurian shale and the soil below the litter layer is acidic, and comprises of clayey to silty loam. Mean rainfall is 166 mm $month^{-1}$ (1990–2000) with a mean temperature of 5.1°C (1984–2000) during the months of study (November–April) at Gwenffrwd-Dinas, <3.5 km from Nantrhydifor (*pers. comm.* D. Anning, Royal Society for the Protection of Birds).

Aquatic decomposition of twigs took place in six streams at Llyn Brianne, mid-Wales, UK (52°08' N, 3°45' W). This location is home to one of the longest running investigations into freshwater acid deposition (see Durance & Ormerod 2007; Ormerod & Durance 2009). Three circumneutral streams were used (pH > 6.9; G2, 52°06'09" N, 3°51'20" W; L6, 52°07'57" N, 3°43'18" W; and L7 52°07'41" N, 3°43'40" W), along with three acidic streams (pH 4.9–5.4; L1, 52°09'48" N, 3°44'32" W; L3, 52°08'31" N, 3°44'00" W; and L8, 52°07'29.61" N, 3°44'48" W).

6.3.3 Litter bag construction

Two randomly-selected twigs from the same year (2011 or 2012) and same CO_2 treatment (ambient or elevated CO_2) were inserted into 15 × 5 cm mesh bags (1 × 1 mm aperture), along with an embossed plastic identification label. One twig per bag was weighed (± 0.01 g) and marked by tying a short piece of fishing line around it. This was used to determine dry-mass loss over the course of the experiment. The remaining twig was used for chemical analyses.

For the terrestrial decomposition experiment, 24 twig bags of each CO_2 treatment were constructed. One bag of each CO_2 treatment was attached 20 cm apart along nylon threads (0.25 mm diameter, 3.5 kg tensile strength; Maxima Fishing Lines, Germany), tied to 0.5 m steel rods (as for Chapter 4) and placed on the surface of the litter layer The experiment ran from 02 November 2012 to 01 May 2013 (182 days).

For the aquatic experiment, 48 twig bags of each CO_2 treatment were constructed. Four twig bags – two of each CO_2 treatment – were attached to metal cages (32.5 × 10.5 × 8.5 cm, aperture 2 × 2 cm) with plastic cable ties. Four of these cages were submerged and secured with 0.5 m steel rods along 10 m reaches in each of the six

study streams. Bag positions were randomised within each cage. The experiment ran from 8 August 2012 to 01 May 2013 (268 days).

6.3.4 Chemical analyses

Along with the experimental twigs dedicated for chemical analysis, three twigs of each CO_2 treatment were set aside for assessment of initial chemical values, and stored at –80 °C until the end of the experimental period. At this time, all twig samples were individually immersed in liquid nitrogen and ground coarsely using a pestle and mortar. Fine powder was then produced by ball-milling the samples (120 s at 50 beats s^{-1} in a Pulverisette 23 ball mill, Fritsch GmbH, Idar-Oberstein, Germany). The percentage dry mass (% DM) of carbon and nitrogen were determined simultaneously by flash combustion and chromatographic separation of approximately 1.5 mg of twig powder, calibrated against a standard ($C_{26}H_{26}N_2O_2S$) using an elemental analyser (Elemental Combustion System 4010 CHNS-O Analyzer, Costech Analytical Technologies, Inc., Milan, Italy). Lignin content was expressed as the percentage of acetyl-bromide-soluble lignin in the Dry Cell Walls (% DCW) of each twig, following the acetyl bromide spectrophotometric method of Foster *et al*. (2010). C/N and lignin/N ratios were calculated.

6.3.5 Mass loss

All twigs allocated for mass loss analysis were rinsed with deionised water before being dried (50°C for 48 hrs) and weighed (± 0.01 g). The proportion of mass remaining was calculated as $1–[(M_t–M_0)/M_0]$ and the decay rate constant (k) per year was calculated using the exponential decay model (Petersen & Cummins 1974), $365(–\ln(M_t/M_0)/t)$, where M_0 is the initial mass (g) and M_t is the mass (g) at time t (days). The biological half-life ($t_{1/2}$; time to 50% mass loss) was calculated as $\ln(2)/k$.

6.3.6 Data analysis

All analyses were undertaken using *R* version 3.0.1 (R Development Core Team 2013). All models were checked graphically for normality and homogeneity of variance (Crawley 2007) and were minimised following a stepwise deletion procedure of non-significant terms ($P < 0.05$) to obtain a minimum adequate model. Planned comparisons of least-square means (LSM; lsmeans function, lsmeans package, Lenth 2013) were performed between the levels of each significant term remaining in the models.

In the terrestrial experiment, separate linear models were fitted for the response variables of carbon, nitrogen and lignin concentrations, and C/N ratio, with atmospheric treatment, days of exposure, and their interaction as explanatory variables. In the aquatic experiment, separate general linear mixed effects models (GLMM; lme function, nlme package, Pinheiro *et al.* 2013) were fitted for carbon and nitrogen concentrations, and C/N ratio, with fixed main and interactive effects of atmospheric treatment, days of exposure and stream pH, and a random effect of stream identity. Initial lignin concentrations were compared using a two-tailed *t*-test, and lignin concentrations after 268 days were compared using a GLMM with atmospheric treatment and stream pH as fixed interactive effects, and stream identity as a random effect.

The proportion of mass lost in the terrestrial experiment was analysed using a GLMM with atmospheric treatment as a fixed effect and rod number nested within block as the random effect structure. A GLMM was also fitted to the proportion of mass lost in the aquatic experiment, using atmospheric treatment and stream pH as fixed interactive effects and cage identity nested within stream identity as the random effect structure.

6.4 Results

6.4.1 Chemical composition

In the terrestrial study, the initial carbon concentration of twigs increased after 182 days of exposure ($F_{1,10} = 9.72$, $P = 0.011$; Fig. 6.1a). The lignin concentration of elevated-CO_2 twigs was 15% higher than ambient-CO_2 twigs ($F_{1,8} = 12.81$, $P = 0.007$; Fig. 6.1b), but this effect was diminished through time ($F_{1,8} = 12.38$, $P = 0.008$; Fig. 6.1b): elevated-CO_2 twigs had a higher initial lignin concentration than ambient-CO_2 twigs (LSM = 4.3%, $P = 0.005$; Fig. 6.1b), but no other pair of atmospheric treatments and time periods differed in lignin concentration ($P > 0.05$). In the aquatic study, the initial lignin concentration of elevated-CO_2 twigs was 65% higher than ambient-CO_2 twigs ($t_4 = 2.94$, $P = 0.042$; Fig. 6.2d). Twig carbon ($F_{1,65} = 50.39$, $P < 0.001$; Fig. 6.2a) and nitrogen ($F_{1,63} = 4.31$, $P = 0.042$; Fig. 6.2b) concentrations were higher after 268 days of exposure compared to initial values, and the C/N ratio was lower ($F_{1,65} = 7.18$, $P = 0.009$; Fig. 6.2c).

6.4.2 Mass loss

Decay rates (k) were 0.05–0.091 year^{-1} in the terrestrial study and 0.194–0.277 year^{-1} in the aquatic study, resulting in biological half-lives of approximately 8–14 and 3 years, respectively (Table 6.1). Ambient-CO_2 twigs had a greater proportion of mass remaining after 182 days of decomposition on the forest floor compared to elevated-CO_2 twigs ($F_{1,23} = 28.13$, $P < 0.001$; Fig. 6.3b). The proportion of mass remaining after 268 days of stream exposure was also higher for ambient-CO_2 compared to elevated-CO_2 twigs ($F_{1,65} = 18.65$, $P < 0.001$; Fig. 6.3a), with no effect of stream pH ($F_{1,4} = 4.71$, $P = 0.096$).

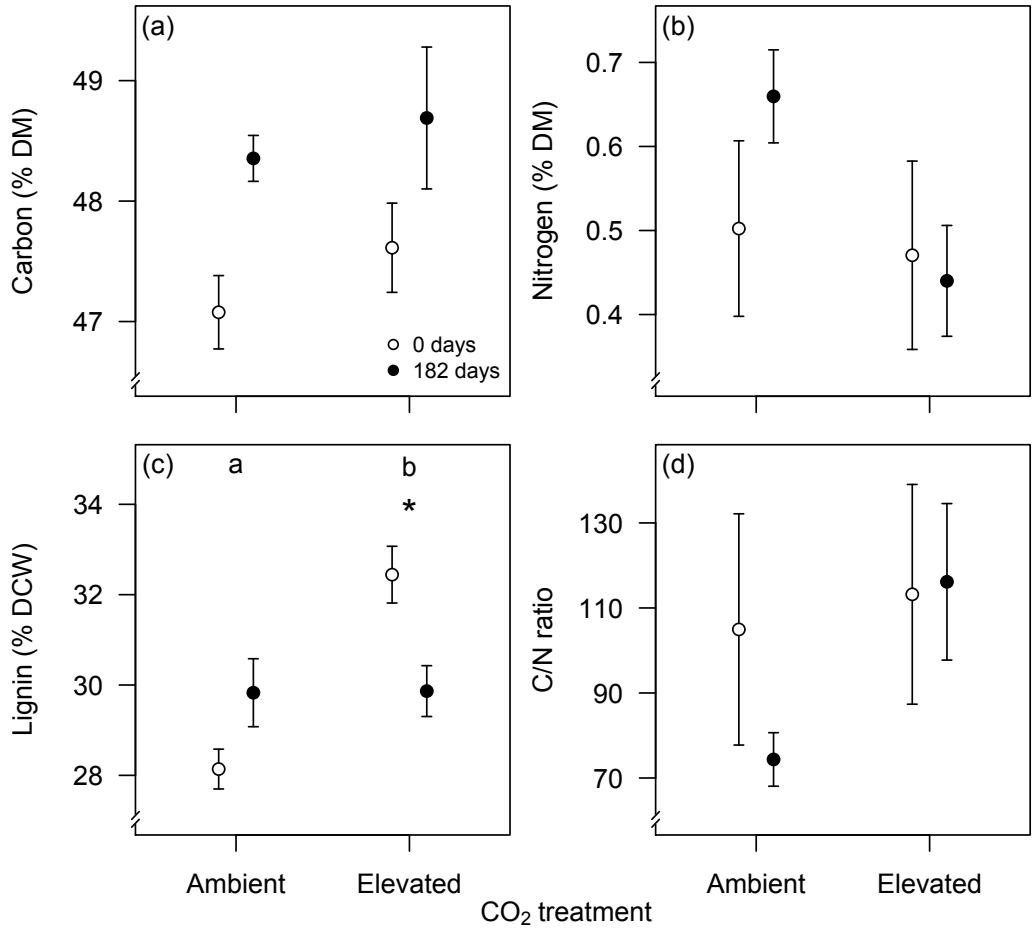

Fig. 6.1. Effect of CO_2 treatment on twig chemical composition following exposure to a temperate deciduous forest floor. Plots show the responses of (a) carbon, (b) nitrogen, and (c) lignin concentrations, and (d) the C/N ratio (DM = Dry Mass, DCW = Dry Cell Wall). An asterisk indicates a significant difference ($P < 0.05$) between time periods within a CO_2 treatment.

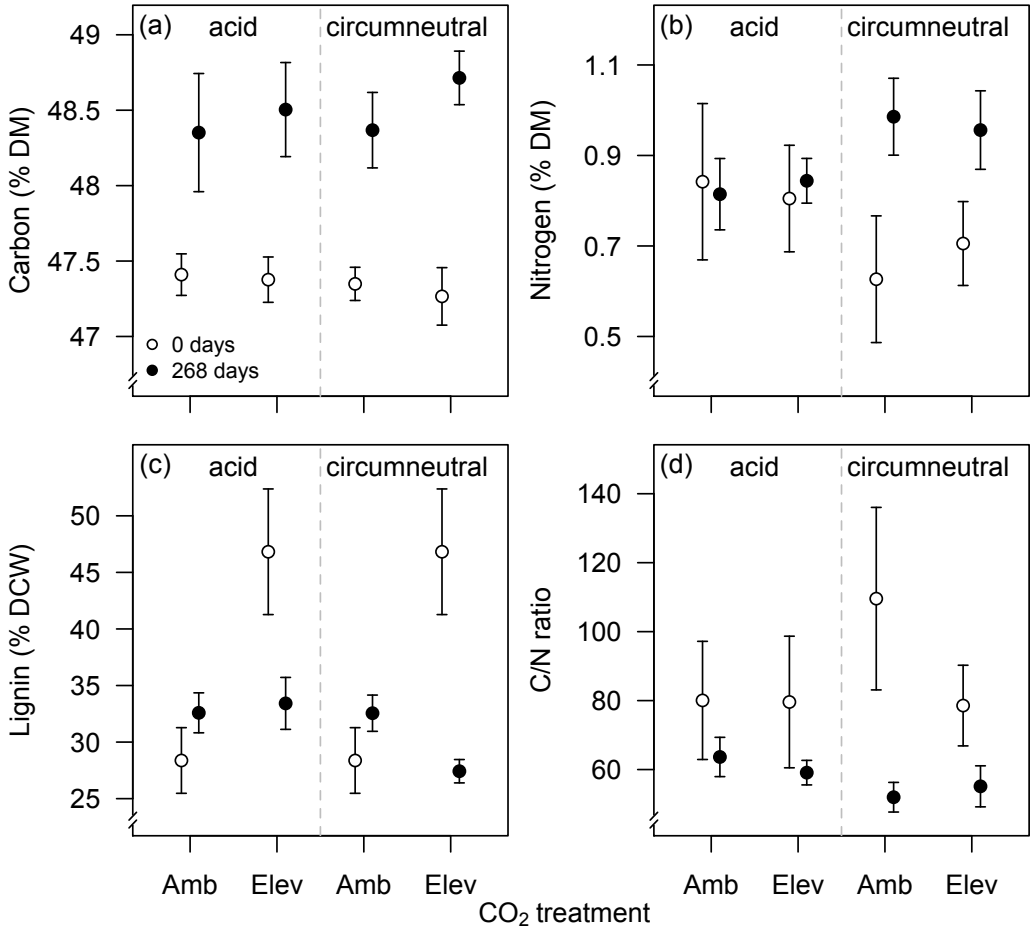

Fig. 6.2. The effect of CO_2 concentration on chemical composition of twig litter exposed to streams of differing pH. Plots show the responses of (a) carbon, (b) nitrogen, and (c) lignin concentrations, and (d) the C/N ratio (DM = Dry Mass, DCW = Dry Cell Wall).

6.5 Discussion

Woody litter is an important carbon and nutrient store in woodlands and adjacent streams, and provides a resource for many organisms. These roles could be disrupted via altered chemical composition and decay caused by atmospheric change and acidification. This study showed that elevated CO_2 increased lignin concentration, that stream pH influenced the change in nitrogen concentration through time, and that mass loss was greater in elevated-CO_2 small woody debris (SWD). These findings suggest that the process of SWD breakdown could be affected by changing

atmospheric composition, and that local habitat conditions could also result in changes to nutrient dynamics.

Table 6.1. Breakdown characteristics of experimental twig litter, including the exponential decay constant (k) and time to 50% mass loss ($t_{1/2}$).

Habitat	Factors	Levels	k (year^{-1})	$t_{1/2}$ (years)
Terrestrial	CO_2	Ambient	0.050	13.81
		Elevated	0.091	7.66
Aquatic	CO_2	Ambient	0.216	3.21
		Elevated	0.277	2.5
	pH	Acid	0.227	3.05
		Circumneutral	0.260	2.66
	pH × CO_2	Acid × Ambient	0.194	3.56
		Acid × Elevated	0.265	2.62
		Circumneutral × Ambient	0.234	2.96
		Circumneutral × Elevated	0.286	2.42

Atmospheric CO_2 treatment altered twig nutritional quality by increasing lignin concentrations, providing some support for Hypothesis 1. Initial lignin concentration was greater in elevated- than ambient-CO_2 twigs, although values were above the normal range for woody stems (16–32%; Chave *et al.* 2009). Increased lignin concentration is typical for woody plants, according to a meta-analysis by Norby *et al*. (2001), although Cotrufo and Ineson (2000) found a 12% drop in the lignin concentration of *Fagus sylvatica* L. twigs. The extra lignification of elevated-CO_2 twigs could have been related to carbon availability – as it was in in a study by Richet *et al*. (2012) – although carbon concentrations were unaffected in the current experiment. The difference in initial lignin concentration between ambient- and elevated-CO_2 twigs was lost by the end of both experiments, unlike a study by Díez *et al*. (2002), which found that the disparity remained for *Q. robur* L., *Alnus glutinosa* (L.) Gaertn. and *Pinus radiata* D. Don branches after three years of stream exposure. Conversely to lignin, carbon and nitrogen concentrations were unaffected by CO_2 treatment in the current study. Similarly, the woody carbon concentration of *Populus tremuloides* Michx. and *B. papyrifera* Marshall was unaffected by CO_2 treatment (Kostiainen *et al.* 2008), as was nitrogen in a study of six deciduous species (Williams

et al. 1986), although nitrogen concentration was reduced in the stems of *Castanea sativa* Mill. seedlings (El Kohen, Rouhier & Mousseau 1992). Effects may not have been observed due to the limited duration of the experiments. For example, twigs may have been expected to gain nitrogen due to incorporation of microbial tissues into the chemical analyses (Dangles *et al.* 2004; Krauss *et al.* 2011), but there may have been insufficient time for differences in colonisation to emerge.

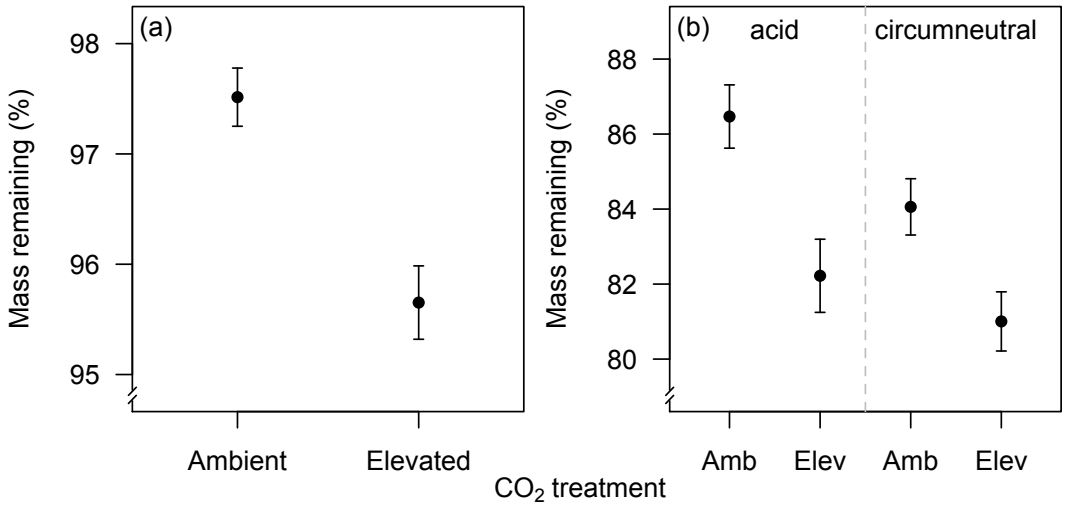

Fig. 6.3. Effect of CO_2 treatment on mass loss of twigs exposed to (a) a temperate deciduous woodland floor for 182 days and (b) to headwater stream environments of contrasting pH for 268 days.

The proportion of mass remaining was greater in ambient- than elevated-CO_2 twigs, which was the opposite result to that predicted by Hypothesis 2. This is unusual, as a greater lignin concentration tends to result in slower decay of organic material (Freschet, Aerts & Cornelissen 2012). For example, a study of wood-chips in large streams found that higher lignin concentrations reduced the breakdown rate, while high lignin/N ratios slowed decay in low order streams (Melillo *et al.* 1983). Despite this, CO_2-induced chemical changes do not always result in altered decomposition rates; the study by Cotrufo and Ineson (2000) found that the nitrogen and lignin content of *F. sylvatica* twigs was reduced under elevated CO_2, but mass loss on a woodland floor was unaffected. In contrast to the effect of CO_2 treatment on mass loss, stream acidity had no effect and provided no support for Hypothesis 3. Organic material has, however, been shown to break down slowly in acidified streams: *F.*

sylvatica leaves, for example, broke down as much as 20 times slower in acidified than in circumneutral streams (Dangles *et al.* 2004). It is possible that the effects of stream acidification may not take effect until beyond the end of the current study, when only approximately 17% of mass had been lost.

Decay rates in the terrestrial study were comparable to coniferous twig decay rates (*k*) of 0.055–0.062 $year^{-1}$ in Colorado, USA (Taylor *et al.* 1991), but lower than rates of 0.14–0.24 $year^{-1}$ in Washington, USA (Edmonds 1987), suggesting that inter-site differences may be more important in influencing breakdown than CO_2 treatment. Aquatic decay rates (*k*) were within the typical range of 0.02 to 0.45 $year^{-1}$ for woody debris, as reviewed by Spänhoff and Meyer (2004). Notably, twigs in the current study broke down faster than SWD with low surface-area-to-volume ratios, supporting Spänhoff and Meyer's (2004) assertion that greater surface-area-to-volume ratios equates to faster decay. Aquatic decay rates were an order of magnitude faster than for terrestrial decomposition, regardless of CO_2 treatment; this may be a result of increased abrasion and leaching in stream environments (Treplin & Zimmer 2012). Slower decay of twigs in both aquatic and terrestrial ecosystems under elevated atmospheric CO_2 could increase residence time of SWD in forests and streams. This could result in extended substrate and nutrient availability to wood-associated biota (Anderson *et al.* 1978; Harmon *et al.* 1986; Tedersoo *et al.* 2003; Berg & McClaugherty 2008) and may increase the role of SWD in habitat modification (Harmon *et al.* 1986; Flores *et al.* 2011; Xu, Liu & Sayer 2013) and nutrient retention (Bilby & Likens 1980; Bilby 1981, 1984; Webster & Tank 2000; Xu, Liu & Sayer 2013).

This study has shown that elevated CO_2 and stream acidification can influence concentrations of lignin and nitrogen in SWD, while mass loss appears to be linked to CO_2 treatment alone. Such effects must be considered alongside other factors implicated in woody decomposition, including tree species (Díez *et al.* 2002; Spänhoff & Meyer 2004), water chemistry (Gulis *et al.* 2004), and stream order (Melillo *et al.* 1983), as well as other global change factors, such as increased anthropogenic activity (Aristi *et al.* 2012). Research in this area is crucial, as the process of SWD decomposition is important to the storage and cycling of carbon and nutrients, and the organisms that use this material as a resource. As such, alterations

to woody debris functioning could result in unpredictable consequences for ecological interactions in both terrestrial and aquatic woodland environments.

7. General discussion

7.1 Synthesis

7.1.1 Overview

Studies in this thesis fulfilled the aims to investigate (i) the effects of elevated CO_2 and urban pollution on the chemistry of both leaf (Chapters 3, 4 and 5) and woody (Chapter 6) litter, (ii) the responses of terrestrial and aquatic invertebrate detritivores to CO_2-treated leaf litters (Chapter 3), and (iii) the decomposition of these litters in terrestrial (Chapters 4 and 6) and aquatic woodland environments (Chapters 5 and 6). An attempt has been made to fill wider knowledge gaps as identified by the literature review (Chapter 2). This includes a greater understanding of (i) the effects of rural and urban locations on litter chemistry and subsequent decomposition (Chapters 4 and 5), (ii) effects of acidification in combination with effects of atmospheric pollution on litter chemical composition and decomposition (Chapters 5 and 6), (iii) a more comprehensive study of invertebrate feeding responses to litter with chemical composition altered by elevated CO_2 (Chapter 3), and (iv) the effect of elevated CO_2 on the chemical composition and decomposition of small woody debris in terrestrial and aquatic habitats (Chapter 6). Although complex, the findings expand on our current understanding of multiple environmental stressors on litter chemical composition and the key ecosystem function of decomposition.

7.1.2 Chemical composition and dynamics

Changes to litter chemical composition were recorded in each experiment, but the direction of these responses was not consistent. For example, urban litter was of higher quality (i.e. lower C/N) than rural litter in Chapter 4, but the opposite was true of Chapter 5 (Table 7.1). CO_2 enrichment generally reduced quality – as found in a meta-analysis by Norby *et al*. (2001) – but this was not true of *Alnus glutinosa* (L.) Gaertn. leaf litter or *Betula pendula* Roth twig litter. This suggests that both inter- and

intra-species-specific effects may be more important in defining litter chemical composition than atmospheric CO_2. While differences in chemical composition occurred, the response of each chemical variable was not always consistent between experiments. For example, the carbon concentration of rural litter was higher than urban litter in the terrestrial leaf decomposition study (Chapter 4), but there was no difference in the aquatic leaf decomposition study (Chapter 5). Urban litter in these two chapters was sourced from separate locations (Cardiff and London), implying that inter-site differences could be responsible (e.g. different pollution levels, climate, etc.). As a further example, litter nitrogen concentration was higher in ambient- than elevated-CO_2 leaf litter in the leaf decomposition studies (Chapters 4 and 5), but the opposite was true of twig material (Chapter 6). This suggests that the chemical composition of litter from different plant tissues is affected differentially by tree growth conditions. Unlike the effects of growth condition, changes to chemical composition through time were relatively consistent across experiments: nitrogen concentration increased, and C/N ratio and phosphorus concentration decreased (Table 7.1). Overall, this work shows that ongoing CO_2 enrichment and urban pollution can alter the nutritional quality of leaf litter and Small Woody Debris (SWD).

7.1.3 Mass loss

Litter mass was lost through time in all experiments, but different experimental conditions varied in their effects on this process. For leaf material, there was little difference in the Ash-Free Dry Mass (AFDM) of ambient- and elevated-CO_2 litters through time, whereas urban litters lost mass faster than rural litters (Table 7.2). Chemical composition appeared to be related to differences in breakdown, as found previously in both terrestrial (Freschet, Aerts & Cornelissen 2012) and aquatic (Ostrofsky 1997) systems. This was, however, not true of leaf litter in the aquatic experiment (Chapter 5), as differences in chemical composition did not result appear to affect mass loss. For twig litter, CO_2 enrichment altered chemical composition by increasing lignin concentration and resulted in faster breakdown. This was unexpected, as higher lignin concentrations generally indicate greater resistance to breakdown (Melillo *et al.* 1983; Cornwell *et al.* 2008; Freschet, Aerts & Cornelissen 2012).

Results for twig litters must, however, be interpreted with caution given the short nature of the study. It is perhaps surprising that an effect of CO_2 treatment was actually found for twigs, given that so much mass remained (96–97% in the aquatic study and 81–86% in the terrestrial study). This study did show that, at least in the short-term, CO_2-enriched twig litter breaks down faster than ambient-CO_2 twigs. These results indicate that differences in growth condition can result in changes to mass loss, and that the relationship between litter chemistry and breakdown is highly variable.

Table 7.1. Summary of changes to litter chemical composition in response to growth condition (GC), conditioning type (CT), stream pH (pH) and time period (T, days). All litter was composed of *Betula pendula*, or *Alnus glutinosa* where marked with †. Litter was composed of leaves (Chapters 3–5) and twigs (Chapter 6). Litters produced in the Controlled Environment Facility (CEF) and Free-Air Carbon Enrichment facility (FACE) were composed of ambient- and elevated-CO_2 material; litters produced *in situ* were composed of rural and urban material. For chapter 6 results, superscripts indicate the experiment location (Te = terrestrial, Aq = aquatic). Asterisks indicate the level of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Non-significant responses are excluded for brevity. Where a factor has more than two levels, planned contrasts took place (marked with asterisks where significant).

Chemical	Factors	Sig.	Direction of difference	Origin	Chapter
Carbon	GC	***	Rural > Urban	*In situ*	4
	CT	*	Aquatic > Terrestrial	FACE	3
	pH	*	Acid > Circumneutral	*Ex situ*	5
	T	*	28 > 0	CEF	4
		**	28 > 0	*In situ*	4
		**	0 > 28	*Ex situ*	5
		*	182 > 0	*Ex situ*	6^{Aq}
		***	268 > 0	*Ex situ*	6^{Te}
	pH × T	*	0 > 28 (Circumneutral**), Acid > Circumneutral (28***)	*Ex situ*	5
	GC × pH × T	*	0 > 28 (Urban, Acid**)	*In situ*	5
Nitrogen	GC	***	Ambient > Elevated CO_2	FACE	3
		***	Rural > Urban	*In situ*	4
		*	Ambient > Elevated CO_2	*Ex situ*	5
		***	Urban > Rural	*In situ*	5
		**	Elevated > Ambient CO_2	*Ex situ*	6^{Aq}

Table 7.1 (continued)

Chemical	Factors	Sig.	Direction of difference	Origin	Chapter
	CT	***	Terrestrial > Aquatic	FACE	3
	pH	**	Acid > Circumneutral	*Ex situ*	5
	T	**	28 > 0	*Ex situ*	4
		*	28 > 0	*In situ*	4
		***	28 > 0	*Ex situ*	5
		***	28 > 0	*In situ*	5
		*	268 > 0	*Ex situ*	6^{Te}
	GC × CT	***	Ambient > Elevated CO_2 (Aquatic***, Terrestrial***)	FACE	3
		*	Elevated > Ambient CO_2 (Terrestrial*)	FACE	3†
	GC × pH	*	Acid > Circumneutral (Urban**)	*In situ*	5
	GC × T	*	Urban > Rural (0***, 28***); 28 > 0 (Rural*, Urban***)	*In situ*	5
		**	0 > 182 (Elevated CO_2*), Elevated > Ambient CO_2 (0**)	*Ex situ*	6^{Aq}
Phosphorus	GC	***	Urban > Rural	*In situ*	4
		***	Elevated > Ambient CO_2	*Ex situ*	5
	T	***	0 > 28	*Ex situ*	5
		***	0 > 28	*In situ*	5
		*	0 > 28	*Ex situ*	4
		***	0 > 28	*In situ*	4
	GC × T	**	Urban > Rural (0***, 28**), 0 > 28 (Rural*, Urban***)	*In situ*	4
Lignin	GC	*	Elevated >Ambient CO_2	FACE	3
	T	*	0 > 28	*In situ*	4
		**	0 > 28	*Ex situ*	5
		*	0 > 28	*In situ*	5
	GC × T	*	Elevated > Ambient CO_2 (0*)	*Ex situ*	6^{Te}
		*	0 > 28 (Ambient CO_2**)	*Ex situ*	4
C/N	GC	***	Elevated >Ambient CO_2	FACE	3
		*	Urban > Rural	*In situ*	4
		**	Elevated > Ambient CO_2	*Ex situ*	5
		***	Rural > Urban	*In situ*	5

Table 7.1 (continued)

Chemical	Factors	Sig.	Direction of difference	Origin	Chapter
	CT	***	Aquatic > Terrestrial	FACE	3
	T	*	28 > 0	*Ex situ*	4
		*	0 > 28	*In situ*	4
		***	0 > 28	*Ex situ*	5
		***	0 > 28	*In situ*	5
		*	0 > 268	*Ex situ*	6^{Te}
	GC × CT	*	Ambient > Elevated (Aquatic***, Terrestrial***)	FACE	3
	GC × pH	*	Rural > Urban (Circumneutral**, Acid***), Acid > Circumneutral (Rural*)	*In situ*	5
	GC × T	*	Urban > Rural (0**), 0 > 28 (Urban**)	*In situ*	4
	GC × pH × T	*	0 > 28 (Ambient CO_2, Acid***), 0 > 28 (Elevated CO_2, Acid***), Elevated > Ambient CO_2 (28, Acid***), Elevated > Ambient CO_2 (0, Circumneutral***), 0 > 28 (Elevated CO_2, Circumneutral***)	*Ex situ*	5

7.1.4 Invertebrates

Invertebrate responses to litter growth conditions were complex, both in terms of feeding (Chapter 3; Table 7.3) and the composition of the assemblage (Chapters 4 and 5; Table 7.3). Prior terrestrial (e.g. Cotrufo, Briones & Ineson 1998) and aquatic (e.g. Ferreira *et al.* 2010) studies have shown that invertebrate feeding is affected by altered litter chemical composition. This only occurred for some species in the invertebrate feeding study (Chapter 3), with little effect on invertebrate assemblages during breakdown on a forest floor (Chapter 4) and in headwater streams (Chapter 5). These results suggest some scale-dependency in both terrestrial and aquatic habitats: effects at the invertebrate species level may not scale up to the community level. It may also be due to the dominance of microfauna in the litter bag studies, compared to the use of macroinvertebrates of the laboratory experiment. The lack of a community-

level effect may also be the result of the presence of species from non-detritivore guilds, which were less affected by changes to litter chemical composition. Beyond effects of CO_2 and urban pollution, consumption of litter was dependent on tree and invertebrate species, along with the habitat of origin (terrestrial and aquatic) of each invertebrate species (Chapter 3). In addition, litter breakdown in streams may be more affected by stream pH than effects of CO_2 and urban pollution on chemical composition (Chapter 5).

Table 7.2. Summary of litter ash-free dry mass changes in response to growth condition (GC) and time period (T, days). All litter is from *Betula pendula*. Litter was composed of leaves (Chapters 3–5) and twigs (Chapter 6). For Chapter 6 results, superscripts indicate the experiment location (Te = terrestrial, Aq = aquatic). Asterisks indicate the level of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Non-significant responses are excluded for brevity.

Factors	Sig.	Levels	Origin	Chapter
GC	*	Ambient > Elevated CO_2	*Ex situ*	4
	***	Rural > Urban	*In situ*	4
	***	Rural > Urban	*In situ*	5
	***	Ambient > Elevated CO_2	*Ex situ*	6^{Te}
	***	Ambient > Elevated CO_2	*Ex situ*	6^{Aq}
T	***	0 > 28***, 28 > 56***, 56 > 112***	*Ex situ*	4
	***	0 > 28***, 28 > 56**, 56 > 112*	*In situ*	4
	***	0 > 28***, 28 > 56***, 56 > 112***	*Ex situ*	5
	***	0 > 14***, 14 > 28*, 28 > 56*	*In situ*	5
GC × T	***	Rural > Urban (28***, 56***, 112***)	*In situ*	4
	**	Rural > Urban (14***, 28***, 56***, 112***)	*In situ*	5
	*	Elevated > Ambient CO_2 (14**), Ambient > Elevated CO_2 (112*)	*Ex situ*	5

7.1.5 Habitat differences

Studies in this thesis considered decay rates in both terrestrial and aquatic environments, although there were no formal comparisons of the two. While similarities exist in the breakdown of litter in these realms (Sinsabaugh *et al.* 1992; Wagener, Oswood & Schimel 1998; Treplin & Zimmer 2012) differences emerge as a result of the influence of stream flow and abrasive action of water, which can speed up the decay process (dos Santos Fonseca *et al.* 2013). Observations in Chapters 4 and 5 reinforced this idea for leaf litter and those of Chapter 6 for SWD, as decay rates were faster in aquatic than terrestrial habitats. Regardless of breakdown habitat, rural litter had the slowest decay rate, followed by urban litter and then ambient- and elevated-CO_2 litters together. Chemical composition also appeared to be affected by the decay habitat. For example, leaf litter chemical composition responded similarly to 28 days' exposure in terrestrial and aquatic habitats in both Chapters 4 and 5 (Table 7.1). Twig chemical composition changed through time in both chapters, but the nature of these changes was dependent on the habitat: nitrogen increased and C/N ratio was reduced through time in the terrestrial study only. This may, however, be a result of differences in the duration of exposure in the terrestrial (182 days) and aquatic (268 days) locations. In Chapter 3 microbial conditioning was shown to affect leaf litter chemical composition differently depending on whether it was exposed to terrestrial or aquatic conditions. Invertebrate feeding responses to this material also seemed to be related to habitat, as aquatic species preferred ambient- to elevated-CO_2 birch discs, but there was no response from terrestrial invertebrate species. Ultimately, repercussions for chemical cycling and invertebrate assemblages may differ between habitat types, but mass loss could remain unaffected.

7.1.6 Litter production site

Leaf litters used in the decomposition studies (Chapters 4 and 5) were collected from trees growing under rural and urban conditions *in situ*, and from *ex situ* trees growing under ambient and elevated atmospheric CO_2 in Cardiff University's Controlled Environment Facility (CEF). Litters produced *in situ* and *ex situ* were not formally compared, but differences in chemical composition, mass loss and invertebrate

assemblages were apparent. These differences may be due to a more optimal growth condition for *ex situ* than *in situ* litters, as the use of potting soil, and a regular watering regime made nutrients and water less limiting in the CEF. Ontogenic factors may also have also played a role, given that litter was collected from mature trees *in situ*, but from saplings *ex situ*. Age and size in temperate deciduous trees can affect not only morphology and phenology (Thomas & Winner 2002; Augspurger & Bartlett 2003; Thomas 2010), but also chemistry. For example, a study of trees ranging from 1–100 cm in diameter by Thomas (2010) found that, in *Tilia americana* L., leaf carbon concentration increases linearly with tree diameter, while nitrogen concentration peaks, and C/N ratio is at its lowest point, at a diameter of approximately 5 cm in *B. alleghaniensis* Britt. and *T. americana*. Although not analysed statistically in the studies contained in this thesis, nitrogen concentrations of mature trees (*in situ*) were lower and C/N ratios higher in comparison with saplings (*ex situ*), and carbon concentrations were higher in rural trees than in both of the *ex situ* treatments. The lower nutritional quality of *in situ* trees may have influenced breakdown, as the remaining AFDM of *ex situ* litters was lower than for *in situ* litters at all time points in both Chapters 4 and 5, indicating consistently faster breakdown of ambient- and elevated-CO_2 litters compared to rural and urban litters.

These findings highlight the need for care when interpreting the results of studies using litter grown under 'artificial' conditions (e.g. greenhouses and closed-top chambers). Alternatives to this method exist, but have their own challenges. In particular, CO_2 can be introduced to trees *in situ*, but there are geographical and financial constraints. For example, some studies make use of litter collected from trees growing near natural CO_2 springs (e.g. Hättenschwiler *et al.* 1997), but these only exist in certain locations and the concentration of gases cannot be controlled. Free-air carbon enrichment facilities (Hendrey & Miglietta 2006) are perhaps the best solution, as they allow for large-scale control of CO_2 inputs to otherwise naturally-growing trees, but they also require a great amount of space and investment (Saxe, Ellsworth & Heath 1998). Financial and practical limitations will likely result in the continuation of 'artificial' conditions in small-scale investigations requiring the control of atmospheric conditions, but the limitations of this approach must be appreciated.

Table 7.3. Summary of invertebrate assemblage responses to growth condition (GC), stream pH (pH) and time period (T, days). Litter was composed of *Betula pendula* leaves (Chapters 3–5) and twigs (Chapter 6). Asterisks indicate the level of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Non-significant responses are excluded for brevity. Note that community analysis does not provide a direction of response, only that there is a difference or not.

Measure	Factors	Sig.	Levels	Origin	Chapter
Abundance	pH	*	Circumneutral > Acid	*In situ*	5
	T	*	56 > 28*	*Ex situ*	4
		**	28 > 112***, 56 > 112**	*In situ*	4
		**	14 > 112*, 28 > 112**	*Ex situ*	5
		**	14 > 112*, 28 > 112***, 56 > 112**	*In situ*	5
Richness	GC	***	Elevated > Ambient CO_2	*Ex situ*	4
	T	*	56 > 112**	*Ex situ*	4
		*	28 > 112**	*In situ*	4
		*	14 > 112*, 28 > 112**	*Ex situ*	5
		**	14 > 112**, 28 > 112***	*In situ*	5
Diversity	GC	**	Elevated > Ambient CO_2	*Ex situ*	4
	T	*	28 > 112**	*Ex situ*	4
	GC × T	*	Urban > Rural (112 **)	*In situ*	5
Community	pH	**	Circumneutral–Acid	*In situ*	5
	T	***	28–112***, 56–112***	*Ex situ*	4
	T	***	28–56*, 28–112***, 56 –112***	*In situ*	4
	T	*	14–56**, 14 –112***	*Ex situ*	5
	T	***	14–112***, 28–112***, 56–112**	*In situ*	5

7.2 Implications

The results of the studies reported in this thesis have several implications for the decomposition of litter following changes to atmospheric conditions and stream pH. For example, expansion of urban areas could result in litter that is susceptible to faster decay, given that urban litter decayed faster than rural litter (Chapters 4 and 5). Conversely, there was little difference in the mass loss of ambient- and elevated-CO_2 leaf litters in either terrestrial (Chapter 4) or aquatic (Chapter 5) locations, suggesting little change to decomposition rates under future CO_2 regimes. Regardless, elevated

atmospheric CO_2 can boost woody plant production (Curtis & Wang 1998; Ainsworth & Long 2005), which could result in a greater amount of leaves. The subsequent build-up of litter on the forest floor could affect several physical factors, such as increased temperature and reduced soil moisture (Xu, Liu & Sayer 2013). Litter stocks could also aggregate in streams, creating anoxic conditions and further disrupting the decay process. Build-ups could also reduce stream flow and encourage habitat formation (Abbe & Montgomery 1996; Beechie & Sibley 1997). This scenario would result in increased carbon and nutrient storage in both terrestrial and aquatic realms.

Invertebrate species composition may also be altered as a result of changes to litter growth conditions and nutritional quality. For example, in the invertebrate feeding study (Chapter 3), differences in the responses of macroinvertebrate species to elevated-CO_2 litter were highlighted. Those responding positively to elevated-CO_2 litter (e.g. *Gammarus pulex* L. and *Odontocerum albicorne* Scopoli fed *A. glutinosa*) could outcompete those that show a neutral or negative response, altering their relative abundances. Generalist species, such as the freshwater amphipod *G. pulex* (Moog 2002), may also be able to take advantage of additional food sources. Sympatric species, such as the woodlice *Porcellio scaber* Latreille and *Oniscus asellus* L., are able to operate in similar niches with slight differences in their dietary needs (Zimmer & Topp 2000); changes to litter nutritional quality could therefore affect the relationship between these organisms. These changes to invertebrate assemblage structure can influence decay, as greater species richness of invertebrate leaf consumers is linked to increased litter processing rates in freshwaters (Jonsson & Malmqvist 2000).

Given the importance of litter as the base of food webs (Moore *et al.* 2004; Hagen *et al.* 2012), alterations to chemical composition and availability could affect multiple trophic levels in both terrestrial and aquatic habitats. This includes microbial decomposers, such as fungi and bacteria (Abelho 2001; Berg & McClaugherty 2008), which in turn can influence invertebrate assemblages. Non-shredder invertebrates in streams will also be affected by changes to the quantity and decay of litter. For example, filtering invertebrates depend on fine particulate organic matter, which could reduce in quantity with reduced decay rates. Altered invertebrate assemblages

in both terrestrial and aquatic locations could affect the wider food web, as these organisms are important prey for fish, birds and small mammals on woodland floors and in streams.

7.3 Limitations

Litter accumulations vary greatly in structure and content, making it difficult to trace the fate of a given litter sample through time. Litter bags resolve this problem by enclosing material of known mass and composition, while being easy to produce and inexpensive. The litter bag approach is an established method (Abelho 2001; Kampichler & Bruckner 2009), having been pioneered in terrestrial systems in the 1950s and 1960s (Bocock & Gilbert 1957; Shanks & Olson 1961; Crossley & Hoglund 1962), and aquatic habitats in the 1970s (Fisher & Likens 1973; Petersen & Cummins 1974). Criticisms of the approach highlight that litter bags can create an artificially stable microclimate and provide invertebrates with extra protection from predators (Crossley & Hoglund 1962), reducing invertebrate migration (Braioni, Gumiero & Salmoiraghi 2001). The choice of mesh size can also have an effect (Crossley & Hoglund 1962; Stewart & Davies 1989; Bradford *et al.* 2002): small apertures may limit the establishment of larger invertebrates (Petersen & Cummins 1974), but also reduce losses due to physical action (wind or stream flow) that might confound detritivore-driven losses. Invertebrates found in litter bags may also not reflect invertebrate composition in the surrounding habitat (Di Sabatino *et al.* 2014).

Most litter bag studies are short-term. For example, a meta-analysis by Kampichler and Buckner (2009) showed that terrestrial litter bag studies generally last for a year or less. Aquatic litter bag studies generally last less than a year (Abelho 2001), reflecting faster breakdown of leaf material in aquatic conditions (Sinsabaugh *et al.* 1992; Treplin & Zimmer 2012; dos Santos Fonseca *et al.* 2013). The duration of decomposition experiments in the studies reported in this thesis were limited by necessity, given the time constraints inherent in a study of this type (e.g. growing trees, performing multiple experiments). The 112 day period did, however, prove to be a suitable timescale for observing the decay of *B. pendula* leaf material in the locations selected: litter lost up to approximately 50% of AFDM on the woodland

floor (Chapter 4), while some litter bags in the aquatic study were almost empty by the end of the experimental period (Chapter 5). While the majority of mass remained at the end of the twig experiments (Chapter 6), the study was still able to provide information on the early decay of this material, as differences in litter chemical composition and proportion of AFDM remaining were already apparent after the short exposure periods.

7.4 Future directions

Experimental studies reported in this thesis considered single species – both trees and invertebrates – in isolation. This simplicity has allowed for broad underlying principles to be investigated. Future work should seek to expand the number of species used to better mimic natural situations. For example, despite the use of multiple invertebrate and trees species reported in Chapter 3, individuals of each invertebrate species were fed litter of one tree species in isolation (e.g. *Asellus aquaticus* fed *A. glutinosa* separately to *B. pendula*). Given the potential for competition between species for litter resources, it would be more realistic to investigate how altering invertebrate abundance and species diversity might affect, and be affected by, litter mixtures of differing tree species. In addition, leaf litter decomposition of just one species (*B. pendula*) was investigated in Chapters 4 and 5, yet litters on forest floors and stream beds are often composed of a multitude of species. There is evidence that the decomposition of litter mixtures can be complex and non-additive (Hättenschwiler, Tiunov & Scheu 2005; Taylor, Mallaley & Cairns 2007; Lecerf *et al.* 2007; Berglund & Ågren 2012) and influenced by invertebrate diversity (Swan & Palmer 2006a; Sanpera-Calbet, Lecerf & Chauvet 2009), making it harder to predict effects of multiple stressors on litter decomposition.

Pairs of contrasting growth conditions – ambient and elevated atmospheric CO_2, and rural and urban – were used to simulate the effects of environmental change on litter chemical composition in Chapters 3 to 6. Responses may vary along gradients of these environmental variables, so future studies should include a greater number of values to increase the resolution of our understanding. For example, CO_2 concentrations could take any value on the continuum from current (approximately

400 ppm) to future concentrations, with projections suggesting that concentrations could approach 1000 ppm by the end of the century (Collins *et al.* 2013). Emissions of pollutant gases also exist on a gradient from urban to rural areas (Lovett *et al.* 2000).

Microbial biofilms are important contributors to the functioning of headwater streams (Battin *et al.* 2003) and can influence leaf litter breakdown (Rier, Kuehn & Francoeur 2007; Danger *et al.* 2013). It is important to understand how changes to litter chemical composition and stream acidity might affect the ability of biofilms to colonise and develop on this substrate. An attempt to investigate this relationship is reported in Chapter 5, but the study was hampered by methodological problems. For example, in a few cases, biofilms grew on the litter bag surfaces rather than leaf surfaces. The use of loosely bound leaf packs could be used to overcome this issue in future, where no material is used to encase the leaves, but rather a thread is used to hold them together.

The Intergovernmental Panel on Climate Change (IPCC) has predicted reduced precipitation and increased temperatures, along with increased frequency of extreme events (IPCC 2013). These changes are likely to affect litter chemistry and decomposition, and should be considered in future studies. For example, Graça and Poquet (2014) found that changes to water availability and soil nutrients resulted in species-specific changes to leaf litter chemistry, with knock-on effects for stream decay. Beyond chemistry-mediated effects, climate influences the breakdown process directly: a global experiment by Boyero *et al*. (2011) found that warm water temperatures resulted in a switch from detritivores to microbes as the main contributor to leaf litter decomposition, increasing CO_2 production and reducing the breakdown of large recalcitrant litter particles. Climate is also an important determinant of invertebrate-mediated decomposition (Wall *et al.* 2008). For example, saprophagous terrestrial macroinvertebrates, such as millipedes and woodlice, could increase in abundance in response to increased temperatures, but the effect could be negated by drought at low latitudes (David & Handa 2010). Increased temperatures influenced feeding preference, growth rate and mortality of the larval form of the caddis fly *Sericostoma personatum* Kirby & Spence, and may have been a better

determinant of invertebrate performance than changes to litter nutritional quality (Ferreira *et al.* 2010).

7.5 Conclusion

The various studies reported in this thesis investigated multiple stressors – CO_2 enrichment, urban pollution and stream acidification – and their effects on the chemical composition and decomposition of leaf and twig litter. Results were dependent on growth conditions (ambient or elevated CO_2, and rural or urban), time periods, stream pH (acidified or circumneutral), invertebrate species (from both terrestrial and aquatic environments), tree species (*A. glutinosa* or *B. pendula*), plant tissues (leaf or twig litter), and habitats (terrestrial or aquatic). This work furthers current understanding on litter decomposition, but more research is required on a wider range of species (invertebrates and trees); on the effects of gradients of environmental variables on chemical composition and decay; the role of biofilms in the decomposition of litters of differing quality; and interacting effects of atmospheric growth conditions and other climate change factors (e.g. temperature and moisture). It is important to anticipate how human-induced global change processes will affect ecosystem functioning in woodlands and headwater streams, given potential impacts to nutrient cycling and the support of food webs. This will allow for a better understanding of how humans may mitigate or cope with future perturbations to ecosystem service provision.

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