Review

The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling

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ABSTRACT

On million-year timescales, carbonate rock weathering exerts no net effect on atmospheric CO_2 concentration. However, on timescales of decades-to-centuries, it can contribute to sequestration of anthropogenic CO_2 and increase land–ocean alkalinity flux, counteracting ocean acidification. Historical evidence indicates this flux is sensitive to land use change, and recent experimental evidence suggests that trees and their associated soil microbial communities are major drivers of continental mineral weathering. Here, we review key physical and chemical mechanisms by which the symbiotic mycorrhizal fungi of forest tree roots potentially enhance carbonate rock weathering. Evidence from our ongoing field study at the UK's national pinetum confirms increased weathering of carbonate rocks by a wide range of gymnosperm and angiosperm tree species that form arbuscular (AM) or ectomycorrhizal (EM) fungal partnerships. We demonstrate that calcite-containing rock grains under EM tree species weather significantly faster than those under AM trees, an effect linked to greater soil acidification by EM trees. Weathering and corresponding alkalinity export are likely to increase with rising atmospheric CO_2 and associated climate change. Our analyses suggest that strategic planting of fast-growing EM angiosperm taxa on calcite- and dolomite-rich terrain might accelerate the transient sink for atmospheric CO_2 and slow rates of ocean acidification.

Key-words: alkalinity; arbuscular mycorrhiza; chemical weathering; climate change; ectomycorrhiza; soil carbonates; soil pH.
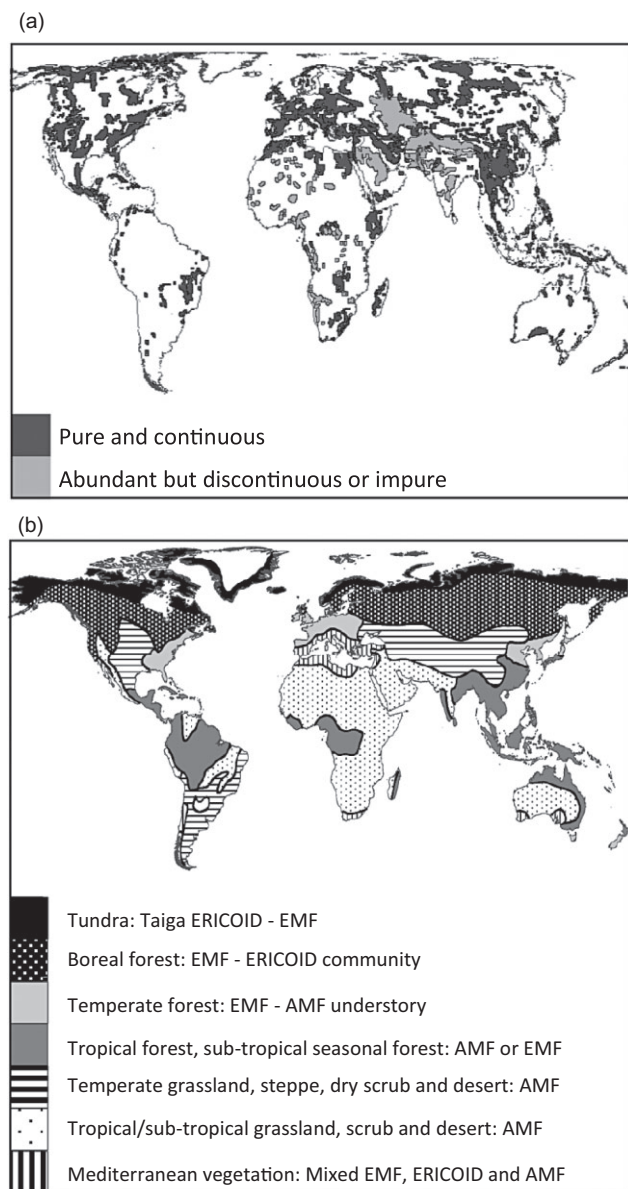
INTRODUCTION

Fossil fuel burning and deforestation have increased the global atmospheric CO_2 concentration by ca. 40% over the past century, an increase of approximately 120 ppm (IPCC 2014). In May 2013, the atmospheric CO_2 concentration measured at Mauna Loa reached 400 ppm (Tans & Keeling 2013), which is probably the highest value for the past 10–20 million years (Beerling & Royer 2011). Planetary surface

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temperatures are responding to the increased radiative forcing from the accumulation of greenhouse gases, with each of the last three decades warmer than any other decade since 1950 (IPCC 2013). Over a timescale of decades-to-centuries, the atmospheric CO_2 concentration is largely regulated by carbon exchanges between the surface carbon reservoirs of the terrestrial biosphere, surface oceans and atmosphere (IPCC 2013). Longer term regulation of atmospheric CO_2 is dominated by the balance between its consumption through rock weathering and release by volcanic degassing (Berner 2003). Although the breakdown of silicate and carbonate rocks are generally regarded as slow processes, recent observational evidence (Gislason *et al.* 2009) and regional-scale modelling (Beaulieu *et al.* 2010, 2012; Goll *et al.* 2014) indicate that CO_2-driven climate change may impact on weathering over timescales as short as decades. Weathering rates of carbonate minerals may be particularly sensitive to contemporary climate change (Beaulieu *et al.* 2012), providing a net sink for atmospheric CO_2 on a decadal-to-century timescale, and a source of continental alkalinity to the surface oceans.

Carbonate lithology (>80% $CaCO_3$) occupies 10.4% of earth's continental surface (Dürr *et al.* 2005) and significant quantities of carbonates are also contained within a variety of sedimentary and silicate lithologies (Hartmann *et al.* 2009). Extensive areas of temperate and boreal forest ecosystems are underlain by carbonate lithologies, which occupy large regions in the Northern Hemisphere between 15–65°N (Fig. 1a,b; Amiotte-Suchet *et al.* 2003; Dürr *et al.* 2005; USGS 2008; Williams & Fong 2010).

However, we know little about the interactions between the roots and symbiotic fungi of N. Hemisphere forests and carbonate rocks. This is despite these regions being particularly important for weathering as, in contrast to the deeply weathered acidic soils of the forested regions of the humid tropics, the soils are often young, developing on fresh regolith deposited at the end of the last glaciation. Their contribution to alkalinity and base cation flux to the oceans will be sensitive to changes in weathering rates, to a much greater extent than older, highly weathered and base-depleted soils. For silicate rocks, it is increasingly well established that root-associated mycorrhizal fungi are the main biotic drivers of rock weathering in soil. These fungi utilize energy from plant photosynthates to grow, proliferate and actively dissolve soil

(a)

Pure and continuous

Abundant but discontinuous or impure

(b)

Tundra: Taiga ERICOID - EMF

Boreal forest: EMF - ERICOID community

Temperate forest: EMF - AMF understory

Tropical forest, sub-tropical seasonal forest: AMF or EMF

Temperate grassland, steppe, dry scrub and desert: AMF

Tropical/sub-tropical grassland, scrub and desert: AMF

Mediterranean vegetation: Mixed EMF, ERICOID and AMF

Figure 1. Global distribution of (a) carbonate rock outcrops (Williams & Fong 2010), (b) major tree–mycorrhizal functional groups (Read 1991; Read *et al.* 2004).
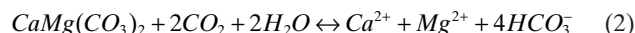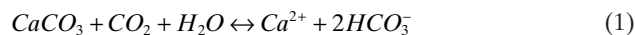
minerals by localized mycorrhizosphere acidification (Casarin *et al*. 2003; Rosling 2003; Rosling *et al*. 2004b; van Scholl *et al*. 2006b). Both of the dominant mycorrhizal functional groups, arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi, are implicated in rock weathering (Koele *et al*. 2014), and actively target the weathering of particular rocks (Leake *et al*. 2008; Quirk *et al*. 2012). Hyphae preferentially colonize minerals like apatite that are sources of inorganic nutrients such as phosphorus, that limit plant growth, and in the process, release calcium and other bases (Blum *et al*. 2002; Hoffland *et al*. 2003; Smits *et al*. 2012). For example, in the Hubbard Brook forest watershed, the weathering of apatite present in the soil has been an important source of alkalinity and calcium ions (Blum *et al*. 2002). This

evidence suggests that although little is known about effects of mycorrhizal fungi in relation to carbonate rock weathering rates, they are likely to be important players in this process.

The aims of this paper are threefold. Firstly, we provide an overview of the role and importance of carbonate mineral dissolution in global carbon cycling and effects of recent land use changes on land–ocean alkalinity fluxes. Secondly, we synthesize evidence for the key mechanisms by which trees and their root-associated AM and EM fungi accelerate the weathering of carbonate rocks. Thirdly, we report results from an extensive field trial designed to investigate the effect of different tree–mycorrhizal functional types on carbonate rock weathering rates. Our field trials include the four major types of carbonate rocks, chalk, limestone, marble and dolomite that underlie Northern Hemisphere forests (Fig. 1a,b). We investigated weathering under a wide variety of mature AM and EM angiosperm and gymnosperm tree species under a common climate and soil at the UK National Pinetum, Kent. Our findings are discussed in relation to the potential contribution of forest ecosystems and plantations to enhance weathering of continental carbonate minerals as part of a wider strategy to promote sequestration of atmospheric CO_2 and reduce the rate of surface ocean acidification.
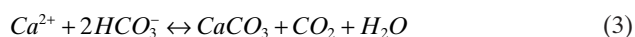
CARBONATE ROCK WEATHERING, GLOBAL CARBON CYCLING AND LAND USE CHANGE

Continental weathering of both carbonate and silicate minerals plays a key role in both the long-term and short-term global carbon cycles and in soil formation. Over millions of years, weathering of silicate minerals regulates the long-term sink for atmospheric CO_2, and therefore climate, as Ca^{2+} and Mg^{2+} ions released from the continents lead to carbonate deposition in the oceans. In contrast, carbonate rock weathering by forested ecosystems is inconsequential for global atmospheric CO_2 concentration on a multimillion year timescale due to the stoichiometry of the reactions. The CO_2 consumed during weathering is balanced by its release back into the atmosphere over $>10^4$ years during marine calcite precipitation (Berner 1992; Liu *et al*. 2011; Zeebe 2012). Generalized weathering reactions for the two main types of carbonate minerals, calcite (Eqn 1) and dolomite (Eqn 2), are given by

$$CaCO_3 + CO_2 + H_2O \leftrightarrow Ca^{2+} + 2HCO_3^- \tag{1}$$

$$CaMg(CO_3)_2 + 2CO_2 + 2H_2O \leftrightarrow Ca^{2+} + Mg^{2+} + 4HCO_3^- \tag{2}$$

Over short timescales of decades-to-millennia, however, carbonate weathering could act as a transient sink for anthropogenic CO_2 and, through land–ocean transport of weathering products, provide a source of alkalinity to the oceans. The CO_2 sink from carbonate dissolution occurs when the rates of the forward and backward reactions in Eqn 1 are unbalanced (Berner *et al*. 1983) and carbonate dissolution on the continents outweighs carbonate deposition in the oceans. This may occur, for example, during the enhanced carbonate weathering predicted in response to

climate warming from a rising atmospheric CO_2 concentration. The duration of CO_2 sequestration may be as short as 10 000 years (Zeebe 2012), and depends mainly on the residence time of ions in the oceans. For HCO_3^-, this is roughly 100 000 years; for Ca^{2+} 750 000–1 200 000 years and for Mg^{2+} > 4 million years (calculated after Berner *et al*. 1983; Li 1982; Ronov 1968). Over geological timescales, far longer than those relevant for the current study, calcite precipitation in the oceans from carbonate derived Ca^{2+} and Mg^{2+} cations re-releases CO_2 and is assumed to balance continental carbonate weathering flux (Eqn 3). Currently, carbonate rock weathering sequesters up to 0.15 Gt C per year for the duration that Ca^{2+} ions remain in solution with bicarbonate, HCO_3^- (Hartmann *et al*. 2013). This equates to half of the total 0.288 Gt C sink from both carbonate and silicate weathering annually (Gaillardet *et al*. 1999).
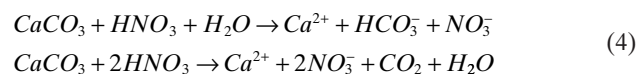
$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O \qquad (3)$$

Carbonate rocks weather an order of magnitude faster than silicate rocks and are responsible for 34–68% of net CO_2 consumption by global-scale continental weathering (Berner *et al*. 1983; Gaillardet *et al*. 1999; Amiotte-Suchet *et al*. 2003; Hartmann *et al*. 2009). Carbonate weathering also provides a vast supply of riverine alkalinity to the oceans. The total flux of dissolved solids originating from land–ocean carbonate weathering is estimated to range from 588×10^6 t year^{-1} to 640×10^6 t year^{-1} (Gaillardet *et al*. 1999; Hartmann *et al*. 2014), although there are still large uncertainties relating to the behaviour of highly active weathering hotspots, and to the strong dependence of carbonate weathering rates on runoff and discharge patterns. Enhanced carbonate rock weathering in response to a warmer climate will drive an increased land–ocean flux of major ions, notably Ca^{2+} and CO_3^{2-} (Zeebe 2012). This enhanced alkalinity flux might partially counteract high CO_2-related ocean acidification by increasing carbonate mineral saturation state and raising surface ocean pH.

Historical studies indicate land use change is a critical factor determining rates of alkalinity fluxes to the oceans. Agricultural practices and land use change cause ecosystem disturbance altering soil hydrology and leading to increased runoff and river discharge from agricultural land (Raymond & Cole 2003; Raymond *et al*. 2008). In North America, fluxes of elements such as Ca are higher from agricultural watersheds in both the upper Mississippi and Ohio River catchments, than from areas with natural vegetation cover, as a result of increased leaching. This is demonstrated in the United States, where conversion of forests to agricultural land has been linked to a dramatic 60% increase in the flux of carbonate alkalinity ($HCO_3^- + CO_3^{2-}$) exported to the ocean from the Mississippi basin in the last 50 years (1954–2001) as a result of both increased run-off and addition of agricultural lime (Raymond & Cole 2003). However, these increased fluxes of alkalinity and associated nutrients such as nitrate and phosphate have severely disrupted the ecology of the Gulf of Mexico where extreme eutrophication has developed, leading to anoxic 'dead zones' that spread up to

15 000 km^2 (Diaz & Rosenberg 2008). Here, subsurface ocean acidity is increased and biogenenic carbonate formation inhibited (Cai *et al*. 2011).

Furthermore, under circumstances such as these, carbonate weathering may be a net source of CO_2 if weathered by an agent not derived from atmospheric CO_2, including nitric and sulphuric acids derived from anthropogenic atmospheric pollution or from nitrogen fertilizers (Perrin *et al*. 2008; Hartmann *et al*. 2013). Additions of nitrogen fertilizers and manures leading to nitrate formation by nitrification processes in the soil, produces nitric acid (HNO_3), which can cause substitution of CO_2-derived carbonic acid for protons from HNO_3 (Eqn 4).

$$CaCO_3 + HNO_3 + H_2O \rightarrow Ca^{2+} + HCO_3^- + NO_3^-$$
$$CaCO_3 + 2HNO_3 \rightarrow Ca^{2+} + 2NO_3^- + CO_2 + H_2O \qquad (4)$$

This practice has produced a deficit in the contribution of atmospheric CO_2 and soil CO_2 to river alkalinity of 7–17% over 30 years in 25 agricultural catchments in the Gascogne region of France underlain by carbonate lithologies (Perrin *et al*. 2008). Globally, such a decrease represents an estimated 1.6–3.8% reduction in CO_2 uptake by carbonate weathering and 2–5% from silicate weathering, and may counterbalance these natural weathering CO_2 sinks. Effects of anthropogenic acids on carbonate weathering as a CO_2 source are even more dramatic in agricultural areas that are concurrently limed and fertilized. This is estimated to release up to 3.35 Tg year^{-1} CO_2 in the United States (West & McBride 2005).

Liming has been used as a strategy to replenish lost calcium, ameliorate surface and ground water acidity and associated aluminium toxicity, to enhance agricultural production and to rejuvenate forests damaged by acid rain in some European and North American regions (Blum *et al*. 2002). Lime is typically added to acid forest soils in the form of calcite or dolomite, and can result in substantially increased carbonate weathering rates (Eqn 1) and subsequent riverine fluxes of carbonate alkalinity (CO_3^{2-}, HCO_3^-), Ca^{2+} and Mg^{2+}.

If the primary reason for forest liming is to combat acidic pollution by sulphuric or nitric acids, then weathering of additional carbonates may not be a CO_2 sink, even if it does improve stream water quality. This parallels the situation with nitrate fertilizer application, as the weathering agent is not derived from atmospheric CO_2 (Eqn 4). Observations in experimental Norway spruce plots in Höglwald Forest, Germany indicate that liming may cause a shift in mycorrhizal community to species with a preference for higher pH, or better calcium tolerance (Nowotny *et al*. 1998). Whether these species achieve tolerance through increasing mycorrhizosphere acidification and therefore, carbonate weathering, is uncertain. Since forests and their associated mycorrhizal fungi are key drivers of mineral weathering (Landeweert *et al*. 2001; Taylor *et al*. 2009), any changes in tree growth and associated soil communities are likely to result in changes to weathering rates, however, this is poorly

documented and should be an area for future forestry research.

Anthropogenic disruption of weathering processes is not limited to forests and agricultural land, but is also occurring in urban soils. In this case, however, human activity may have provided a new CO_2 sink, through enhancing carbonate sequestration via the carbonation of industrial waste materials. An estimated 7–17 billion tonnes of artificial silicate waste materials are produced annually, with the potential to sequester 190–332 million tonnes urban C through precipitation of pedogenic carbonates reacting with oxides in cements and building rubble (Renforth *et al.* 2011). This is a process that reverses the loss of CO_2 to the atmosphere that occurs as limestone is burned to produce cement (Renforth *et al.* 2011; Manning & Renforth 2013), but does not reverse the CO_2 loss associated with the fossil fuel used in making cement. Studies at one brownfield site in Newcastle and former steelworks in Consett, NE England, have shown that carbonation sequesters about 25 ± 12.8 t C ha^{-1} year^{-1} (Renforth *et al.* 2009). In urban brownfield sites, carbonation of industrial waste materials is primarily an abiotic process. However, vegetation planting at these sites would be likely to further increase carbonation rate by increasing soil CO_2 concentrations, which in these carbon sequestering soils, might be rate limiting (Renforth *et al.* 2011; Manning & Renforth 2013).

MECHANISMS OF CARBONATE WEATHERING BY TREE–MYCORRHIZAL PARTNERSHIPS

Mechanisms of mineral weathering by both AM and EM fungi have been reviewed recently in relation to silicate weathering (Rosling *et al.* 2004a; Wallander & Thelin 2008; Taylor *et al.* 2009; Brantley *et al.* 2011). In this section, we highlight three key weathering strategies of mycorrhizal fungi with special emphasis on how they relate to carbonate rocks. (1) Physical weathering by mycorrhizal hyphae penetrating or trenching minerals; (2) chemical weathering by secreted organic acids and chelating cations that acidify the rhizosphere and complex metal cations; and (3) action of respiratory CO_2-derived carbonic acid, and the mycorrhizal secretion of carbonic anhydrase, an enzyme that catalyses production of this acid.

Physical weathering by mycorrhizal hyphae

Both AM and EM fungi radiate from host plant root tips through the soil forming networks of hyphae whose active growing tips proliferate when they encounter rock and mineral particles containing particular elements or minerals (Leake *et al.* 2008). The narrow, 3–10 μm diameter, hyphae of EM fungi can trace the contours of rock particle surfaces, following scratches or ridges and entering rock pores and fissures (reviewed in Hoffland *et al.* 2003; Landeweert *et al.* 2001). Fungal hyphae strongly adhere to mineral surfaces and exert forces, using high turgor pressures. Hyphae can widen existing fissures and grow between mineral cleavage planes and within spaces in the rock.

Hyphae can produce channels and trenches on carbonate minerals, for example on polished marble after 4 months colonization by *Heboloma crustaliniforme* ectomycorrhizal with *Pinus sylvestris* (Rosling 2003). Channels have also been seen on a variety of silicate minerals under both AM and EM forests (Hoffland *et al.* 2003; Smits *et al.* 2005; Quirk *et al.* 2012; Koele *et al.* 2014); on chlorite and biotite in microcosms containing EM tree seedlings in symbiosis with *Paxillus involutus* (Bonneville *et al.* 2009; Gazzè *et al.* 2012), and on biotite flakes incubated in a liquid culture with EM *Suillis tomentosus* (Balogh-Brunstad *et al.* 2008). A recent field study with a limited range of species suggests that the magnitude of mineral weathering and trenching is greater under EM tree species than under AM tree species (Quirk *et al.* 2012). Fungi can tunnel into rocks (Hoffland *et al.* 2003; Hoppert *et al.* 2004; Smits *et al.* 2005; Koele *et al.* 2014), and there are reports of limestone colonized by an endolithic lichen in which the fungal and algal partners live inside the rock (Hoppert *et al.* 2004). However, fungal tunnelling appears to be slow and contributes only modestly to weathering, accounting for around than 2% of total feldspar weathering in a temperate coniferous forest (Smits *et al.* 2005).

Few quantitative experiments have determined the contribution of physical hyphal contact to carbonate mineral weathering. Li *et al.* (2009) used liquid culture experiments, and incubated limestone granules with a saprotrophic *Penicillium* fungus for 25 h, and calculated dissolution rates from measurements of pH and Ca^{2+} concentration. Limestone dissolution was reduced by 22% when the grains were separated from the culture by a fine mesh to exclude *Penicillium* spp. hyphae. Fungal growth in liquid media is often very different to that on complex media like soil and minerals so that extrapolation from such experiments to field relevance presents significant challenges.

Chemical weathering by organic acids and anions

Low molecular weight organic acids (LMWOAs) are potentially the most important biological weathering agent in soil (reviewed in Landeweert *et al.* 2001) because carbonate minerals are highly susceptible to acid dissolution. LMWOAs secreted by plants, fungi and other microorganisms are involved in metal detoxification and mineral dissolution in soils, enlarging and merging surface enlarging and merging surface scratches and linear pits developed through cleavage of minerals for experimental studies (Gazzè *et al.* 2012). LMWOAs are more important than carbonic acid for weathering of carbonates. For example, *Penicillium* spp. grown in liquid culture showed greater reduction in carbonate rock dissolution when organic acids were neutralized using NaOH than when acetazolamide inhibited carbonic anhydrase activity thus severely inhibiting carbonic acid production (Li *et al.* 2009).

LMWOAs, however, form only a minor component of the total complement of organic acids in soil and have a fast turnover rate, being rapidly biodegraded or precipitated in complexes and adsorbed to soil particles (van Hees *et al.*

2002). LMWOAs combine soil solution acidification, via proton release, with the ion-complexing capacity of chelating anions. Chelating species such as LMWO anions have several delocalized negative charges spread across the anion and can surround a small charge-dense cation forming several ionic bonds. Chelation occurs when bonding between the chelating anion and metal cation is stronger than the interaction between the cation and its mineral substrate. Oxalic acid can facilitate carbonate dissolution through forming complexes with both Ca and Mg ions, in addition to a range of other ions such as K, Mn, Zn, Cu, Al and Fe from silicate substrates (Landeweert *et al*. 2001). EM fungi secrete a range of mono-, di- and tri-carboxylic acids, including oxalic, malic and citric acid, dependant on fungal species (Arvieu *et al*. 2003; van Scholl *et al*. 2006a). AM fungi are generally considered to not produce LMWOAs, but a recent study with AM clover plants identified formate and acetate in culture medium surrounding AM mycelium (Toljander *et al*. 2007). However, these monocarboxylates have very limited complexation abilities compared to the di- and tri-carboxylic acids released by EM fungi.

Although in bulk soil solution LMWOAs concentrations are likely to be too low to exert major mineral weathering effects, where roots and mycorrhizal hyphae establish intimate contact with minerals, they may be able to generate sufficiently high concentrations to significantly promote chemical weathering. Typical concentrations range from <0.1–17 μM in the field and <0.1–2.3 μM in laboratory pot experiments (van Hees *et al*. 2002, 2003). The most important dissolution mechanisms of LMWOAs are targeted, local rhizosphere acidification and formation of a layer of organic exudates known as a biofilm (Saccone *et al*. 2012). The range of acids differs between both tree species and mycorrhizal association and include oxalic, citric, butyric, formic, phthalic, malonic, succinic, lactic and tartaric acids (Arvieu *et al*. 2003; Casarin *et al*. 2003; Sandnes *et al*. 2005). Oxalate is probably the most important LMWOA for carbonate dissolution (Gadd & Poole 1999). It is typically secreted at higher rates than other minor LMWOAs, and its release is coupled to soil acidification by H⁺. The coupling of oxalate secretion and mycorrhizosphere acidification is suggested by the correlation of oxalate concentration and pH in liquid culture experiments (Arvieu *et al*. 2003; Casarin *et al*. 2003), and will heighten the impact of this LMWOA on carbonate dissolution.

Many EM fungi produce low molecular weight, iron-chelating ligands called siderophores (Szaniszlo *et al*. 1981), and there are indications that the AM fungal clade *Glomus* may produce siderophores, but this is unproven (Haselwandter 2008). The main function of siderophores is for uptake of iron, reacting with otherwise insoluble Fe(III) to form highly stable complexes in solution (Renshaw *et al*. 2002). When in pure culture, mycorrhizal fungi can produce a wide range of hydroxamate siderophores in response to iron deprivation (Szaniszlo *et al*. 1981; Renshaw *et al*. 2002), an effect stimulated by $CaCO_3$. Siderophores may be important in releasing iron from both calcite and dolomite rocks where iron is a common trace element (Scoffin 1987) and removal of

Fe might facilitate weathering by altering the structural integrity of such rocks. This weathering may be important in calcareous soils where Fe(III) is virtually insoluble, often limiting plant and fungal growth, as evidenced by lime-induced iron chlorosis (Marschner 1995).

Rhizosphere acidification by respiratory CO₂ production and carbonic anhydrase

Both EM and AM fungi may promote carbonate mineral weathering by acidifying the soil solution through proton extrusion associated with cation uptake (Koele *et al*. 2014). Soil respiration and CO_2 production by mycorrhizal fungi result in a weakly acidic solution of carbonic acid (Eqn 5), a reaction that can be up-regulated by the action of carbonic anhydrase (CA) enzyme.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \qquad (5)$$
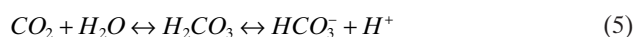
CA catalyses the conversion of soil CO_2 into carbonic acid (Eqn 5), which is the primary abiotic weathering agent of soil minerals. This reaction occurs slowly at ambient CO_2 partial pressures found in the environment and is often the rate-limiting stage in limestone dissolution (Liu *et al*. 2005). Genes for CA synthesis are expressed in EM fungi (reviewed in Landeweert *et al*. 2001) and have also been identified in AM fungi (Tisserant *et al*. 2012). Carbonic anhydrase activity accelerates carbonate dissolution through coupled weathering reactions, so long as reaction end-products continue to be removed through soil drainage (Lian *et al*. 2011). In a closed system starting far from equilibrium, CA addition increases initial carbonate dissolution rates by up to a factor of 50, depending on rock type and grain size (Liu 2001; Liu *et al*. 2005). The effect of CA catalysis depends on the availability of CO_2 substrate, and potentially also on the type of carbonate mineral (Liu 2001; Liu *et al*. 2005). Abiotic kinetic experiments tracking solution conductivity, as a proxy for dissolved Ca^{2+} and Mg^{2+}, illustrated that limestone, but not dolomite, dissolution could be increased tenfold by addition of 0.2 μM bovine CA, but only when solution pCO₂ was greater than 100 Pa. Soil CO_2 concentration varies between ecosystems, and is typically far higher than in the laboratory experiments of Liu *et al*. (2005). For example, in Arctic soils, pCO₂ = 7600 Pa (Alaska); in temperate forest soils pCO₂ = 15 300 Pa (Germany); and in tropical forests pCO₂ = 25 500 Pa (Trinidad; Brook *et al*. 1983). At a field study at various sites across limestone areas in China, Li *et al*. (2005) showed that densely vegetated areas had higher microbial CA activity linked to extracellular CA production by fungi. Although no mycorrhizal fungi were explicitly identified, several plots were located within evergreen broad-leaf and needle-leaf (pine) woodland in which trees universally form mycorrhizal associations implicating the involvement of their mycorrhizal associates. This is supported by the higher soil CA activity detected in the vicinity of plant roots and the sharp decline in CA activity with depth. Further work is required to resolve the contribution of mycorrhizal fungi to

these processes and the extent to which CA increases rates of carbonate weathering in forest ecosystems.

EFFECT OF TREE–MYCORRHIZA FUNGAL PARTNERSHIPS ON RATES OF CARBONATE WEATHERING IN THE FIELD

Previous work suggests that AM and EM trees are likely to enhance the weathering of carbonate minerals to differing extents through organic acid exudation and acidification of their mycorrhizospheres. We therefore undertook field trials on representative tree–mycorrhizal functional groups found on carbonate rock lithology (Fig. 1a,b) and focused on characterizing weathering within single-species stands (30 m^2) of mature trees in a common parent soil at the UK's national pinetum in Bedgebury, Kent, UK (51.07°N, 0.44°E). Replicate trees ($n = 5$) from 13 species (Table 1) were selected to represent the four functional groups: AM gymnosperm, AM angiosperm, EM gymnosperm and EM angiosperm. Crushed and sieved carbonate rock samples (2.5 g with a particle size of 0.5–1.18 mm diameter) were buried in hyphal in-growth bags ($n = 5$ bags per rock per species) planted at 10 cm depth ca. 1 m from the base of each tree. We investigated four carbonate rock types: (1) marble (Carrara, Italy); (2) oolitic limestone (Oxfordian, N. Yorkshire); (3) chalk (Cretaceous, N. Yorkshire); and (4) dolomite (Permian, Doncaster) that span the entire range of carbonate lithologies beneath N. Hemisphere forests. For comparison, we also included in-growth bags with basalt grains (Tertiary, Skye), a carbonate-containing silicate rock that has been previously used in weathering experiments involving trees and mycorrhizas (Leake *et al*. 2008; Quirk *et al*. 2012).

In the present field trials, we estimated net weathering rates by measuring mass loss of rock samples in mesh bags retrieved after 3 months burial during the active growing season (April–August 2013). For comparison between rock types, molar weathering rates were calculated, using experimentally determined rock densities and assuming spherical particle geometry. Mesh bags (35 μm pore size) allow for exchange of substances with soil pore water and permit hyphal in-growth and colonization while excluding roots. To investigate possible linkages between mineral weathering rates and soil acidity, we collected soil samples from the surface organic-rich soil horizon near the base of each tree. Air-dried and ball-milled soil was mixed with water in a 1:1 ratio v/v and pH measured using a pH meter (Jenway 3540 pH meter, Bibby Scientific Limited, Stone, UK; calibrated at pH 4, pH 7 and pH 10) Statistical analyses were performed using R2.14.2, in association with the R-Studio interface (www.rstudio.com 2012). Data were analysed using a variety of statistical techniques including ANOVA, and graphed using the ggplot2 package (Wickham 2009) and GraphPad Prism version 6.04 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com GraphPad 2014).

After 3 months of burial (May–August 2013), rates of mass loss (i.e. net weathering) from calcite-containing rock grains in the root-excluding mesh bags were more than an order of magnitude faster than those from basalt grains (calcite average mass loss 14.0 times higher than for basalt under EM angiosperms, Fig. 2). High carbonate weathering rates are partly determined by the functional type of the tree stand, with greatest calcite-weathering rates beneath EM angiosperms (two-way ANOVA, $F_{1,9} = 9.94$, $P < 0.05$; Fig. 2b). Both tree group (angiosperm/gymnosperm) and mycorrhizal type significantly affected calcite-weathering rate. However, Fig. 2 indicates that this result may be driven by the much greater 3 month weathering rates beneath EM angiosperms. Basalt and dolomite weathering was similar between tree–mycorrhizal functional groups, and showed no significant effect of either tree or mycorrhizal type (two-way ANOVA: basalt, $F_{1,9} = 0.11$, $P = 0.75$; dolomite, $F_{1,9} = 0.89$, $P = 0.37$; Fig. 2b).

SOIL ACIDITY AS A DRIVER OF CARBONATE WEATHERING

At our field site, Bedgebury Pinetum, soil has been developing for almost 100 years beneath the stands of mature trees

Table 1. Tree species used in mycorrhizal weathering field study at Bedgebury Pinetum

Mycorrhiza	Tree group	Leaf habit	Species (where identified)	Family
AM	Gymnosperm	*E*	*Ginkgo biloba*	Cupressaceae
		E	*Metasequoia glyptostroboides*	Cupressaceae
		E	*Sequoia sempervirens*	Cupressaceae
		E	*Araucaria araucana*	Araucariaceae
	Angiosperm	*E*	*Ilex aquifolium*	Aquifoliaceae
		D	*Acer pseudoplatanus*	Sapindaceae
		D	*Liriodendron tulipifera*	Magnoliaceae
EM	Gymnosperm	*E*	*Pinus sylvestris*	Pinaceae
		D	*Larix decidua*	Pinaceae
		E	*Abies nordmanniana*	Pinaceae
	Angiosperm	*E*	*Nothofagus dombeyi*	Nothofagaceae
		D	*Quercus petraea*	Fagaceae
		D	*Eucalyptus coccifera*	Myrtaceae

Tree group: gymnosperm or angiosperm; mycorrhizal type: ectomycorrhizal (EM) or arbuscular mycorrhizal (AM); leaf habit: evergreen (*E*) or deciduous (*D*).
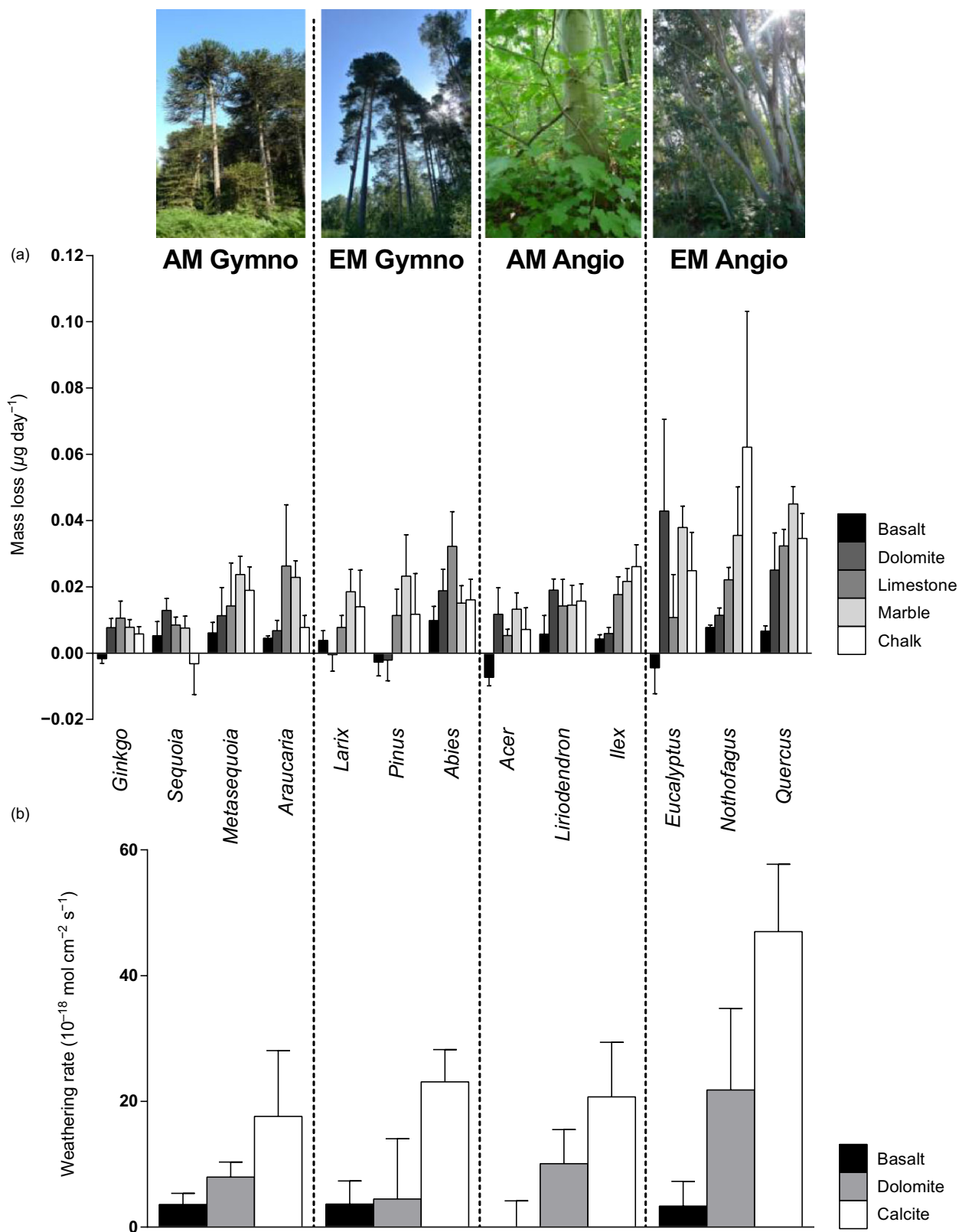
Figure 2. Weathering of rock grains buried in root-excluding mesh bags after 3 months (May–August 2013) at Bedgebury Pinetum, Kent, UK, under representative trees in established stands. Values are mean ±standard error of the mean (SEM) mass loss. Results sorted by (a) tree species (*n* = 3 replicate trees of each of 13 species, Table 1) and (b) tree–mycorrhizal functional group for each calcite (chalk, oolitic limestone and marble), dolomite and basalt minerals (*n* = 3 tree species of each functional group, except arbuscular mycorrhizal (AM) gymnosperm where *n* = 4).
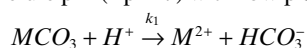
selected for this study and distinct differences are evident beneath different tree species. The most acidic soils were located beneath trees forming EM partnerships (pH 3.50 ± 0.04, $n = 6$; mean $\pm$ standard error of the mean (SEM)). Soils beneath trees forming AM partnerships were less acidic (pH 4.30 ± 0.22, $n = 7$; mean $\pm$ SEM). Although mycorrhizal partnership had a significant effect on soil pH (AM/EM: three-way ANOVA; $F_{1,5} = 8.76$; $n = 13$; $P = 0.032$), there was no effect of either tree type (angiosperm/gymnosperm: three-way ANOVA; $F_{1,5} = 0.08$; $n = 13$; $P = 0.79$) or leaf habit (evergreen/deciduous: three-way ANOVA; $F_{1,5} = 1.92$; $n = 13$; $P = 0.22$). This indicates that rhizosphere acidification associated with the direct and indirect effects of mycorrhizal fungi, and especially EM fungi, may be the most important factor in determining soil pH, and associated weathering. This contrasts with the common expectation that greater acidification occurs in conifer (gymnosperm) forest soils at Bedgebury (Ovington 1953). Soil acidification by both angiosperm and gymnosperm EM trees may be due to proton extrusion, accumulation of humus, loss of base cations through leaching and tree uptake, and the production of organic acids (Arvieu *et al*. 2003; Casarin *et al*. 2003, 2004; Sandnes *et al*. 2005). In our field trials, soil pH was one of the main drivers of calcite rock weathering (Fig. 3; $F_{1,11} = 8.17$; $n = 13$; $P = 0.016$; $R^2 = 0.426$). A similar general pattern of higher mass loss with increasingly acidic soils was also apparent for dolomite (Fig. 3b), but not significant ($F_{1,9} = 2.52$; $n = 11$; P = 0.147; $R^2 = 0.22$).

To understand the geochemical basis for the dependency of field-observed weathering rates on soil pH and proton availability, field data were compared to theoretical weathering rates calculated according to Eqn 6 (Chou *et al*. 1989):

$$Rate = k_1[H^+]^s + k_2[H_2CO_3]^s + k_3 \qquad (6)$$

In Eqn 6, s signifies reaction order, for example, in acidic conditions, the dissolution of calcite used $s = 1$. k_1, k_2, k_3 are temperature-dependant rate constants for the three carbonate weathering reactions below. The relative importance of these three mechanisms depends on the soil pH and pCO$_2$ environment:
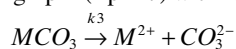
Acidic pH (<pH 7) with low pCO$_2$:
$$MCO_3 + H^+ \xrightarrow{k_1} M^{2+} + HCO_3^-$$

Moderate pH (ca. pH 7) with high pCO$_2$:
$$MCO_3 + H_2CO_3 \xrightarrow{k_2} M^{2+} + 2HCO_3^-$$
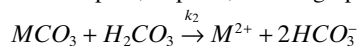
High pH (>pH 7) with low or no pCO$_2$:
$$MCO_3 \xrightarrow{k3} M^{2+} + CO_3^{2-}$$

Calcite, CaCO$_3$, dissolution is first order and $s = 1$, dolomite, CaMg(CO$_3$)$_2$, has a different rate dependence on pH and follows a fractional order when dissolving in acidic conditions, often quoted as $s = 0.5$. Dolomite dissolution also has a different stoichiometry because 2 moles of acid are required to fully dissolve one mole of dolomite. The theoreti-

cal weathering rate for dolomite (Fig. 3) is calculated with $s = 0.75$, for comparison with the work of Chou *et al*. (1989). Temperature-dependant rate constants, k_1, k_2, k_3 from Chou *et al*. (1989) and Palandri & Kharaka (2004) are used to calculate theoretical weathering rates, at both 25 °C and adjusted to field air-temperature (13.4 °C, hourly weather station data, May–August, Bedgebury Pinetum) using activation energy, E_a (J K^{-1} mol^{-1}; Palandri & Kharaka 2004). When calculating the theoretical weathering rate within the pH range 3–6, which spans the pH of all soils sampled at Bedgebury Pinetum in December 2013, it was assumed that the main factor determining weathering rate is the acidic rate term, $k_1[H^+]^s$, and the curve was calculated without taking into account the effect of pCO$_2$, represented by the neutral term $k_2[H_2CO_3]^s$. This latter assumption was made as in the field soil CO$_2$, carbonic acid and organic acid exudates all contribute to the overall, acidic, soil pH and inclusion of an additional $k_2[H_2CO_3]^s$ may result in an overestimation of theoretical weathering rate. In this study, these soil acidifying agents have not been separated, as their relative importance is likely to vary seasonally and in response to climatic conditions, changing soil hydrology and biological activity. With these assumptions, theoretical curves at 298 K lie close to the experimental results of Chou *et al*. (1989) within the pH range investigated in this field study. For dolomite, the theoretical curve lies slightly below the experimental results of Chou *et al*. (1989), and inclusion of the k_2, carbonic acid, term might improve their correspondence.

Experimental field weathering for calcite and dolomite both follow the same pattern as theoretical rate, when adjusted for particle surface area (mean diameter = 0.84 mm). However, carbonate rock weathering under field conditions was several orders of magnitude slower than that expected based on rates derived from laboratory studies (6 for calcite-containing rocks and 2–3 for dolomite), as often observed for silicate weathering rates under field versus laboratory conditions (White & Brantley 2003).

One factor to explain the gap between observed and theoretical dissolution is that in the field, water–mineral contact time is far lower than in laboratory studies. Low water availability, in periods with little rainfall will slow weathering as dissolved ions cannot be transported away from the weathering surface, increasing near-surface saturation and shifting the carbonate dissolution equilibrium to make dissolution less favourable. Rate constants k_1, k_2 and k_3 used to calculate theoretical weathering rates for calcite and dolomite are also likely to be different in the field than in the experimental closed systems used to derive these values. Secondly, the field rate constants will vary slightly depending on the precise mineral composition of the rocks weathering. Although literature estimates of these rate constants for pure minerals provide a reasonable estimate, experimental determination of k_1, k_2 and k_3 may improve correspondence between theoretical and field results.

The surface area vulnerable to weathering action is likely to be another factor contributing to the discrepancy between field and theoretical results. Petrological examinations show chalk, dolomite, marble and limestone have a
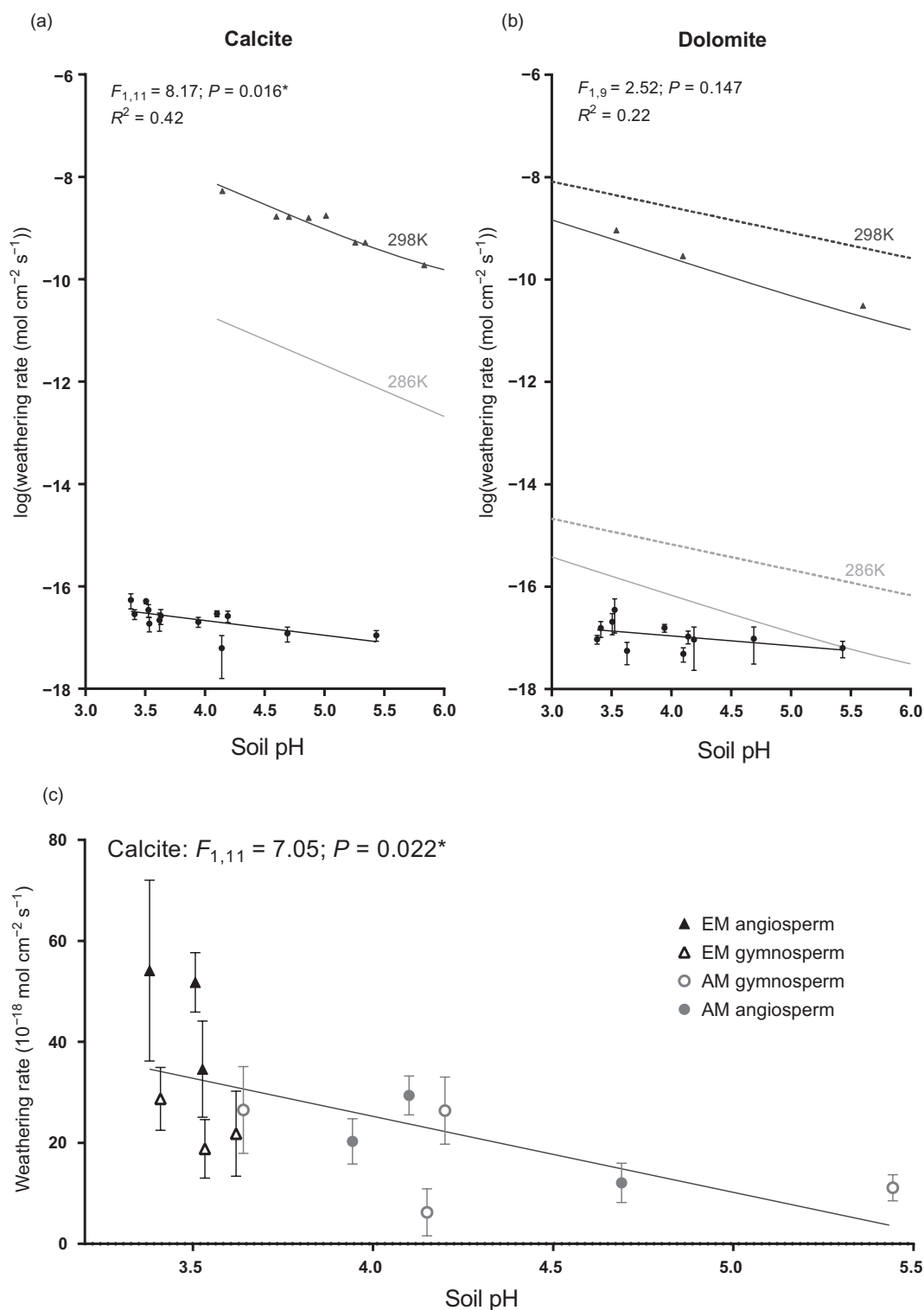
Figure 3. Effect of soil pH on field weathering rates at Bedgebury Pinetum, Kent, UK (May–August 2013). Comparison of experimental results with calculated theoretical rates at 298 K (dark grey lines; dark grey data points are experimental results from Chou *et al.* 1989 at 298 K) and field temperature, 286.4 K (light grey lines) for (a) calcite ($n = 13$ tree species) and (b) dolomite ($n = 11$ tree species), across the measured range of field soil pH. For dolomite, dashed lines signify order of the dolomite dissolution reaction (Eqn 6), $s = 0.5$, and solid lines represent order, $s = 0.75$ as used in Chou *et al.* (1989). Rate constants were adjusted for 286.4 K using the Arrhenius equation and activation energies from Palandri & Kharaka (2004). Outputs of two-way ANOVA for dependence of field weathering rate on pH are displayed on each graph panel. (c) Calcite field weathering rates for each tree species ($n = 13$ tree species, three replicate trees for each species) adjusted for surface area. EM angiosperm (black closed symbols); EM gymnosperm (black open symbols); AM angiosperm (grey closed symbols); AM gymnosperm (grey open symbols).

wide range of textures and properties (Scholle & Ulmer-Scholle 2003), and quantification of surface area and porosity for the carbonate rocks used in this experiment may help to reduce the gap between field experiment and theoretical results. Laboratory weathering rates are often normalized using BET (Brunauer-Emmett-Teller) surface area from nitrogen gas absorption measurements (Brantley & Mellott 2000; White & Brantley 2003; Navarre-Sitchler & Brantley 2007), although in the current study, the surface areas of both theoretical and experimental rock grains are calculated based on spherical particles as BET values for the rocks used are not available.

DIRECT EVIDENCE OF FUNGAL–CARBONATE INTERACTIONS WEATHERING

We observed surface weathering features on dolomite grains, excavated after 3 months burial in the field, using SEM imaging (Philips XL-20 SEM, Philips, Eindhoven, Netherlands; Department of Biomedical Sciences; University of Sheffield) that avoided pretreatment before gold sputter-coating of samples. SEM imaging of grains recovered from beneath mature stands of *P. sylvestris* and *Larix decidua* indicate that the hyphae form a mesh-like network across the grain surface (Fig. 4a). On some grains, SEM imaging revealed a series of grooves or channels (Fig. 4b) where hyphae have either been dislodged or have deflated *in situ*. In Fig. 5b, the surface of the rock is more granular. Here, hyphae grow across the surface, and younger, branching, hyphae of smaller diameter appear to disappear into the rock matrix.

Evidence of hyphal channelling on the surface of carbonate rocks has been shown previously on polished marble in microcosm studies with *Heboloma crustuliniforme*, EM with pine seedlings (Rosling 2003). In our samples, SEM imaging revealed trenching features on dolomite along the path of a hypha that has been partially detached from the rock surface (Fig. 5c,d). The diameter of hyphae observed on rock grains in stands of both *Pinus* and *Larix* trees have a diameter of approximately 3 μm, within the typical range for EM hyphae (Hoffland *et al.* 2003) and of slightly greater width than the 2 μm channel identified in Rosling (2003). Where hyphal channels were observed, the texture of the adjacent rock surface was found to be pitted. Surface etching may extend slightly beyond the area occupied by a fungal hypha, which may indicate the involvement of fungal secretions, such as organic acids (Saccone *et al.* 2012), in the formation of these weathering features (Fig. 5d). The degree of surface etching by hyphae might depend on rock type and surface structure at the point of hyphal adhesion, as although, in the current study, images of dolomite show clear pitting (Fig. 5d), comparison with marble (Rosling 2003) shows little surface texture modification within the hyphal channel.

Hyphae growing on carbonate rock grains are occasionally encrusted with angular crystals (Fig. 4a); due to secondary mineral formation. These crystals are likely to be calcium oxalate as recently found for EM fungal growth on a range of minerals (Schmalenberger *et al.* 2010). The hyphae of oxalate-producing fungi are often encrusted with Ca-rich oxalate crystals, observed in *Piloderma* (Arocena *et al.* 2001; Casarin *et al.* 2003), *Paxillus involutus* (Lapeyrie *et al.* 1987) and on non-mycorrhizal fungi on weathering chalk (Verrecchia & Dumont 1996). These distinctive crystals are formed from the weathering products of Ca-bearing soil minerals (Kolo *et al.* 2007).

HOW MIGHT TERRESTRIAL CARBONATE WEATHERING RESPOND TO RISING CO$_2$ AND ANTHROPOGENIC CLIMATE CHANGE?

Our results support the expectation that trees and mycorrhizal fungi play an important role in accelerating carbonate rock weathering under field conditions. The potential sensitivity of rates of carbonate rock weathering in response to rising atmospheric CO$_2$ concentrations and climate change has been addressed through coupled modelling of

(a)

(b)
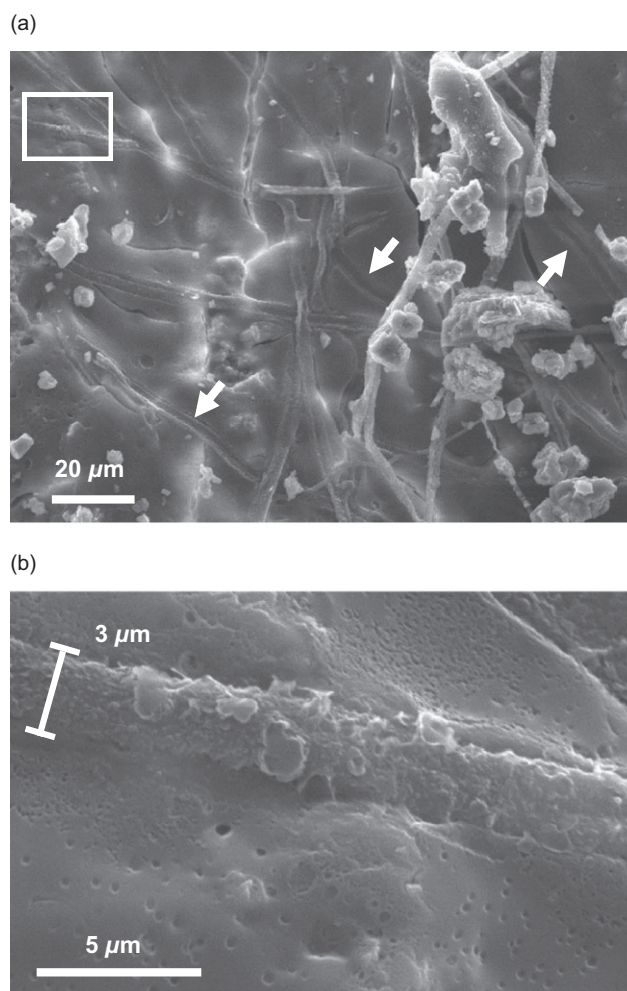
Figure 4. (a) SEM images of hyphae growing across the surface of a dolomite grain buried under *L. decidua*. Arrows indicate possible channels where hyphae have either detached from the rock surface, or have deflated *in situ*. Area highlighted is enlarged in b. Close up of surface detail and hypha channelling on dolomite grain. Scale bar = 3 μm and indicates typical diameter of an EM hypha.
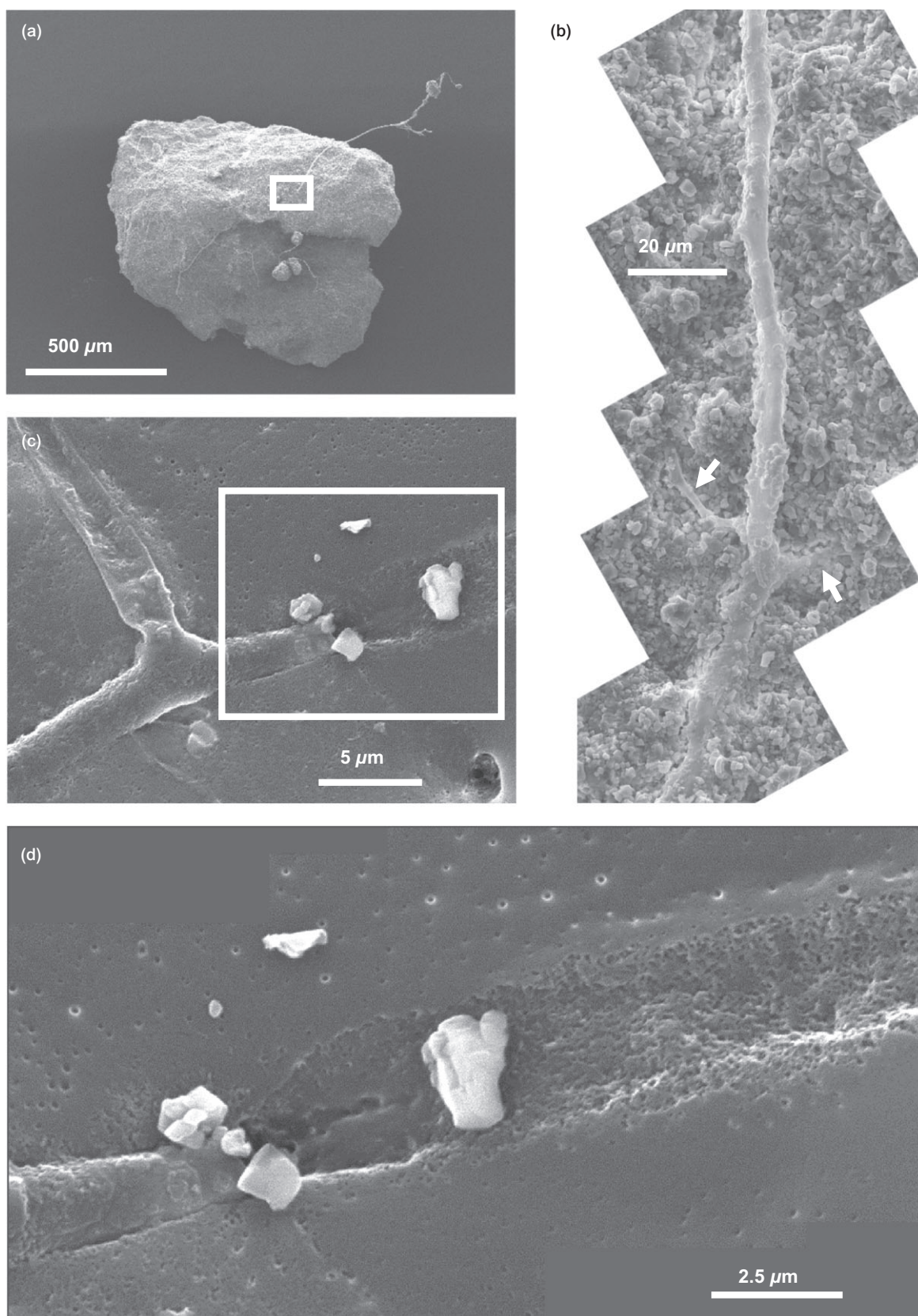
Figure 5. (a) SEM images of hyphae growing across the surface of a chalk grain buried under *P. sylvestris*. Area highlighted is enlarged in c. (b) Arrows indicate the location of smaller diameter branching hyphae. (c) Channelling by hypha on the surface of a dolomite grain buried under *L. decidua*, close-up of surface texture detail enlarged in d.

vegetation–soil weathering interactions for arctic ecosystems. Beaulieu *et al.* (2012) reported a detailed modelling analysis of silicate and carbonate rock weathering in the Arctic Mackenzie River Basin watershed, in response to an increase in the atmospheric CO_2 concentration from 355 to 560 ppm and associated climate changes. Their model predicted an increase in bicarbonate export from carbonate weathering by 18% and from silicates by 40%, with watershed bicarbonate flux from carbonate dissolution remaining an order of magnitude higher than that from silicate weathering. However, climate change predicted for the Mackenzie watershed is greater than the global average change, and other studies predict that CO_2-driven intensification of weathering may be less dramatic. Earlier modelling work of Beaulieu *et al.* (2010) estimated a much smaller, 2.4–5%, increase in silicate weathering per 100 ppm rise in atmospheric CO_2 since 1850, across a range of catchments spanning different lithologies and climatic zones. Goll *et al.* (2014) predicted a 13% increase in global chemical weathering per 100 ppm CO_2 over the same period, yet also demonstrated that there is still much uncertainty relating to CO_2-driven weathering intensification. Weathering responses to elevated pCO_2 vary spatially, and the magnitude and global pattern of responses is model dependent. Increases in the weathering-derived bicarbonate flux at elevated CO_2 are related to changes in runoff and temperature, CO_2-fertilization of vegetation net primary production (DeLucia *et al.* 1999) and to higher rates of autotrophic and heterotrophic respiration (Allen *et al.* 2000; Andrews & Schlesinger 2001).

The direct effects of elevated CO_2 on plants can affect soil hydrology, through partial stomatal closure reducing transpiration and improving water use efficiency (reviewed in Ainsworth & Rogers 2007). As a consequence, soil drainage is increased, along with removal rate of weathering products, thus maintaining Eqn 1 in disequilibrium. This is supported by modelling results of Godderis *et al.* (2013) that predict changes in soil drainage will be the most important factor affecting dolomite weathering in the Mississippi valley, if CO_2 were to increase from 315–700 ppm over the period 1950–2100. Dissolution of dolomite responds linearly to drainage, as the equilibrium of carbonate alkalinity is maintained (Eqn 1). Overall, in the modelled watershed, CO_2 consumption from carbonate weathering is predicted to decrease, over the period 1950–2100, mainly because of a 4 °C temperature increase, as the solubility of dolomite, and other carbonate rocks, decreases with temperature (Godderis *et al.* 2013).

Few studies have investigated the responses of mineral weathering to increasing atmospheric CO_2 concentrations experimentally. However, in the Duke FACE (free-air CO_2 enrichment) experiment, loblolly pine trees (*P. taeda* L.) were fumigated with CO_2 elevating atmospheric concentrations above ambient by 200 ppm for over 2 years and soil mineral weathering responses quantified. Both autotrophic and heterotrophic respiration, and associated soil CO_2 concentrations, increased over this period, leading to a corresponding decrease in soil pH. Increased soil acidity provided a more favourable environment for dissolution of carbonates and other minerals (Andrews & Schlesinger 2001), and

resulted in a 271% increase in soil cation concentration, increased soil alkalinity and 33% higher flux of dissolved inorganic carbon within groundwater. Studies of mycorrhizas in the Duke forest showed that EM fungi proliferated with host trees exposed to CO_2 fertilization. However, there was no increased investment in AM fungi of understorey vegetation or AM saplings including *Acer rubrum* (Garcia *et al.* 2008). Although the Duke FACE site overlies clay-rich soil derived from igneous silicate rock, a similar but potentially more dramatic increase in weathering may be expected for carbonate-containing soils. This is due to the strong dependence of carbonate weathering on carbonic acid (Eqns 1, 5), and their faster weathering kinetics. Overall, modelling studies and field experiments illustrate a variety of weathering responses to increasing atmospheric CO_2. These range from greatly accelerated carbonate dissolution, right through to decreased weathering rates in some catchments. Future carbonate weathering responses are likely to be highly heterogeneous, depending on the precise combination of vegetation, geology, soil drainage and climate change within each watershed, making this a critical area for future research.

CONCLUSIONS

Temperate and boreal forest ecosystems overlie much carbonate lithology, particularly in the Northern Hemisphere and historical land use changes are already exerting substantial effects on carbonate rock weathering rates and land–ocean alkalinity fluxes. This review shows that there are plausible physical and chemical mechanisms involving mycorrhizal fungi that could accelerate carbonate rock weathering within forest soils. Evidence from our ongoing field study at the UK's national pinetum confirms a wide range of mature EM and AM angiosperm and gymnosperm tree species increase the weathering rates of carbonate rocks. Preliminary results for 3 months rock incubation (May–August 2013) show differences in calcite and dolomite weathering rates when exposed to different tree and mycorrhizal functional groups. Especially rapid weathering is observed within EM angiosperm forest soils. The cause of these observed differences requires further investigation, but in line with theoretical expectations, soil pH appears to be a central factor in determining carbonate dissolution rates. Within our field trial, we also observed direct interactions between fungal hyphae and weathering rock grains within EM forest soils. These interactions act to alter mineral surface topography and increase weatherable surface area through fungal trenching and pitting at the hyphal-mineral interface. Fungal channelling is likely to result from a combination of physical, chemical and enzymatic weathering mechanisms, and future studies of these features may help improve understanding of the fine-scale mechanisms of fungal carbonate weathering.

More broadly, our findings are relevant to enhancing carbonate weathering, CO_2 sequestration and potential amelioration of surface ocean acidification. Our analyses suggest strategic planting of suitable tree–mycorrhizal plant func-

tional groups, such as notable fast-growing EM angiosperm taxa, might offer a possibility for utilizing weathering as a natural ecosystem service to accelerate these effects.

ACKNOWLEDGMENTS

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