Growth and soil carbon dynamics of short rotation coppice species (Willow and Poplar) under variable nutrient and water availability

Leigh-Anne Kemp

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Declaration

I declare that the thesis presented is my own work, except where references are made to other research and that it has not been submitted, in whole or in part, in any previous application or award for a higher degree elsewhere. Contributions by other researchers are properly acknowledged

Leigh-Anne Kemp
Lancaster University, October 2023

Abstract

Short rotation coppice (SRC) bioenergy crops are an attractive option to reduce anthropogenic greenhouse gas (GHG) emissions from primary energy production and agriculture. Fast-growing, high-yield SRC crops sequester large amounts of carbon in biomass, require minimum input, and can be grown on marginal land. However, we do not know how the carbon dynamics of SRC crops will be affected by future climate changes. Given that crops grown on marginal land often require nutrient additions and are subject to large fluctuations in soil moisture, I assessed the potential for inoculation with arbuscular mycorrhizal fungi (AMF) and fertilization to mitigate or exacerbate the effects of extreme weather events (drought and flood) on the growth, biomass, and soil GHG emissions of two SRC crop species (willow and poplar). I hypothesised that fertilization and AMF inoculation would mitigate the effects of extreme weather events on crop growth and biomass, but that nutrient addition would increase soil GHG emissions after drought and during flooding. My mesocosm experiments demonstrated that biomass increased by 26% with AMF inoculation and by 56% with nitrogen (N) addition. However, AMF inoculation stabilised soil CO_2 emissions during a drought-rewetting event, whereas N-addition resulted in higher soil CO_2 emissions during rewetting and after the drought. Furthermore, N-addition boosted soil CO_2 and methane (CH_4) emissions during flooding. Importantly, soil CO_2 or CH_4 emissions during flooding were higher in N-fertilized trees with a history of drought, and drought resulted in lower leaf biomass at the start of the subsequent growing season. The body of work presented in this thesis provides strong evidence that fertilization of SRC crops could increase soil GHG emissions during extreme weather events, and highlights promising new avenues for investigation into AMF inoculation as a potential alternative to boost SRC crop yield and resilience to drought.

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I dedicate this thesis to my daughter Erin, because of you I will forever strive to be the best version of myself.

COVID-19 Impact Statement

The research work conducted during this PhD was disrupted by the COVID-19 global pandemic. Disruption was encountered due to COVID-19 restrictions that included restricted access to polytunnels and other experimental or analytical facilities. As a result, the planned experiments were shortened and simplified to ensure they could be conducted with the available time and resources. Importantly, planned measurements of methane for Chapter 3 were dropped due to lack of access to the necessary equipment, and experimental plans for Chapters 3 and 4 were altered to account for the possibility of further restrictions; the studies had to be significantly reduced in size to allow for them to be completed in windows of relaxed restrictions and before the end of the growing season. In addition, travel restrictions delayed access to the analytical laboratory at Lancaster Environment Centre, which further reduced the number of samples and analyses possible. Thus, planned analyses of microbial biomass and inorganic nitrogen, which require fresh samples, were not possible. Intended analysis of soil microbial community composition, as well as repeat measurements of root biomass and mycorrhizal colonisation were abandoned due to limited site access and compound delays to the experimental work.

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1. Introduction

1.1 Global carbon dynamics

Substantial research now links the acceleration of increased global mean temperatures, leading to climate change, to the increase in greenhouse gas (GHG) emissions released by anthropogenic activity. Carbon dioxide (CO_2) emissions have increased from 2 billion tonnes in 1990 to over 36 billion tonnes by 2015 (Roser and Ritchie, 2020). The use of fossil fuels is the greatest contributor to atmospheric GHGs, followed by the combined practices used in modern agriculture and forestry (Pachauri and Meyer, 2014). The intensification of agricultural practices has also led to a decline in soil health, a loss of soil, and loss of biodiversity, which could feed back to climate change because healthy soils represent the largest terrestrial carbon store (Seidu, 2017). Globally, soils contain two to three times the amount of carbon than the atmosphere (Davidson, Trumbore and Amundson, 2000) and the soil carbon stock is declining due to increasing global temperatures, degraded soils, and soil erosion (Buckingham, Rees and Watson, 2014). However, rising atmospheric CO_2 has also increased the soil carbon sink in some temperate forests, and increased nitrogen inputs can aid carbon soil sequestration by boosting biomass production (Buckingham, Rees and Watson, 2014). The drivers of change in soil carbon stocks can thus range in severity depending on season and location. The increase in the global human population has placed further pressure on soils as a natural resource, creating competition for fertile agricultural land (Davidson, Trumbore and Amundson, 2000). Consequently, agricultural practices and land management play a significant role in whether soil acts as a carbon net source or sink (Buckingham, Rees and Watson, 2014).

To combat the increasing risks associated with climate changes and increasing pressures on natural resources, many countries are now looking to developing carbon-neutral practices for sustainable farming and energy generation. In 2019, the UK became the first major economy to pass legislation committing to net zero GHG emissions by 2050 (UK Government, 2019). One focus of the UK government is to produce carbon-neutral energy, growing all areas of the renewable energy sector. The drive for renewable energy sources includes increased biomass production as an energy source to power existing coal-fired energy stations and biomass power accounted for 13% of UK energy production in 2020 (Department for Business Energy & Industrial Strategy, 2021). Biomass energy refers to any non-fossilised biological energy source (Field, Campbell and Lobell, 2008), and is considered advantageous over other renewable resources because it is a consistent source of energy, providing both a regular income to landowners and a sustainable energy supply, which offers short to mid-term options where other resources fail (Rowe, Street and Taylor, 2009). A particularly promising approach to biomass generation is short-term rotation coppice, whereby fast-growing woody species are coppiced at regular intervals to yield biomass for energy production, but their root stocks are left in the ground for up to 30 years (McKay, 2011). A meta-analysis by Djomo et al (2011) found that short rotation coppice (SRC) has an energy yield 14-86 times higher than coal with 9-161 times lower GHG emissions. In addition, SRC crops have the potential to sequester and store large amounts of carbon from the atmosphere during photosynthesis and growth (González-García et al., 2014), making them a highly attractive alternative to fossil fuels. The most promising SRC crops in the UK are willow (*Salix* spp.) and poplar (*Populus* spp.), with willow being the most popular at present. In the UK, SRC crops are usually harvested every three years once established, however plantations can be coppiced as little as every five years (Aylott et al., 2008).

1.2 Short rotation coppice

Crops for climate change mitigation

Due to their high carbon and energy content, SRC crops for bioenergy are recognised as a sustainable alternative to fossil fuel derived energy while also contributing to climate change mitigation targets (Oliver, Finch and Taylor, 2009). To meet the UK's 2050 decarbonisation targets, a minimum of 14% of UK primary energy demand must be met by bioenergy crops such as SRC *Salix* and *Populus* plantations (Department for Environment Food & Rural Affairs, 2020). Short rotation coppice can achieve carbon neutrality by the time the first harvest is undertaken (usually after three years) and remains carbon neutral for up to 20 years (Amichev et al., 2012). After five years, SRC crops sequester significant amounts of atmospheric CO_2, increasing stock by up to 15%, although this can depend on historical land use, where converted arable soils will see an increase in storage, land converted from grassland has a neutral carbon storage, while deforesting to create SRC can lead to an initial decrease in carbon storage (Harris, Spake and Taylor, 2015; Rytter, Rytter and Högbom, 2015; Richards et al., 2017). By contrast, the creation of conventional forestry can take decades to achieve the same carbon sequestration levels (Amichev et al., 2012). Hence, the relatively short time SRC crops require to achieve a carbon neutral state highlights the importance of these crops in achieving carbon neutral energy production (Amichev et al., 2012). Importantly, SRC crops can also enhance soil carbon sequestration. The stabilisation and storage of soil organic carbon (SOC) is a slow process taking up to 50 years depending on land use and location (Harris, Spake and Taylor, 2015; Milner et al., 2016). Estimations show that conversion from annual to perennial crops can influence SOC for 30-50 years in temperate regions, while there is very limited data on land change use from forest to biomass crops (Harris, Spake and Taylor, 2015). Although the lack of long-term data on land conversion to SRC does impair the ability to infer long term carbon stock increases, depending on historical land use, the current data show a significant short-term increase in soil carbon for arable land converted to SRC (Harris, Spake and Taylor,2015). The extent of carbon sequestration by SRC crops is often related to historic land use and soil condition, with abiotic conditions and geographical location also impacting crop yield (Aylott et al., 2008).

Besides carbon sequestration and storage, SRC crops might also contribute to mitigating emissions of methane (CH_4) and nitrous oxide (N_2O), the two most potent GHGs after CO_2 especially when converting from historically agricultural land (Drewer et al., 2017). Methane has a global warming potential around ten times greater than CO_2 (IPCC, 2014) and nitrogen inputs such as fertilizers alter nitrogen cycling processes and boost soil N_2O emissions (Wang et al., 2014a). In addition, nitrogen fertilisation and leaching can influence CH_4 cycling (Butterbach-Bahl et al., 2002; Zhang et al., 2017), making soils an important contributor to rising atmospheric GHG concentrations (Venterea et al., 2003). However, SRC crops such as *Salix* spp. not only have lower fertiliser requirements than many other agricultural crops (depending on soil quality) (Caslin, Finnan and McCracken, 2011) but can also take up and store large amounts of nitrogen, thus reducing leaching and fertiliser-related GHG emissions such as N_2O and CH_4 (Drewer et al., 2012).

Other benefits of short rotation coppice crops

Short rotation coppice bioenergy crops offer many ecosystem benefits besides carbon storage and GHG reductions, including soil decontamination and improvement of soil health and structure (Lockwell, Guidi and Labrecque, 2012). Although SRC crops require large areas of land (Rowe, Street and Taylor, 2009), *Salix* and *Populus* SRC crops have a high tolerance for water-logged conditions but are also able to withstand short-term drought more effectively than many traditional arable crops in the UK, making them ideal for marginal land, including areas of intense flooding (Dimitriou, Busch and Jacobs, 2009). In addition, willow and poplar SRC crops are hardy and require little maintenance after establishment (Aylott et al., 2008).

Establishing SRC crops on agricultural land and maintaining a 3–5-year harvest cycle can increase species richness and abundance (Rowe, Street and Taylor, 2009; Vanbeveren and Ceulemans, 2019; Weih, Glynn and Baum, 2019). As SRC crops offer habitat for many natural enemies (predators, parasitoids) of crop pests, they can also reduce pesticide application to nearby conventional crops (Piotrowska, Czachorowski and Stolarski, 2020). In addition, SRC crops are viable for up to 20 years requiring no tillage and infrequent use of agricultural machinery, which reduces soil bulk density (Lockwell, Guidi and Labrecque, 2012) and disturbance to soil organisms, increasing overall soil health (Rowe, Street and Taylor, 2009). The breakdown of soil fungal networks through the removal of annual crops and tillage can lead to soil erosion, loss of soil nutrients, and increased GHG emissions (Paz-Ferreiro and Fu, 2016), all of which are reduced or avoided in SRC crops. Finally, bioenergy crops are typically coppiced after senescence has occurred, and the leaf litter is left in-situ, which reduces the need for nutrient additions and provides ground cover to help improve weed control (Amichev et al., 2012), while leaving the soil undisturbed. However, it is important to note that the benefits of SRC crops are highly dependent on former land use. Improvements to soil health and biodiversity are far more likely to be seen on land which has previously been used for conventional arable agriculture, while replacing previously unimproved grasslands or pasture often yields little improvement (Rowe, Street and Taylor, 2009).

Establishment and management of SRC bioenergy crops

Although biomass production is the second largest renewable energy supply, (Buck et al., 2020), the current area set aside for SRC production in the UK amounts to less than 1% of the fertile agricultural land (Department for Environment Food & Rural Affairs, 2020). However, SRC crops can be planted on marginal land to reduce competition with food production; this strategy would increase the land available to grow bioenergy crops, while also increasing income for landowners (Amichev et al., 2012). Nonetheless, SRC crops must maintain consistently high yield to offer financial incentive over other crops or the use of fossil fuels as a primary energy source (Oliver, Finch and Taylor, 2009) and therefore, growing crops on marginal land often requires a more intense fertilisation regime to produce enough yield (Schweier and Becker, 2013; Aronsson, Rosenqvist and Dimitriou, 2014a; Fabio et al., 2018). Despite this, SRC is classed as a low input crop: weed control is only required during the establishment period (one to two years), fertilisation is recommended every three years after a harvest and the soil is only tilled before planting, which can be as little as every 20 years (Amichev et al., 2012).

In contrast to most annual crops, SRC crops require little fertilisation: during the first year of establishment, the recommendations in the UK are to add no additional nutrients to the soil (Agriculture and Horticulture Development Board, 2019). However, this does not consider land use legacy. Growing SRC crops for bioenergy production on marginal or degraded agricultural land could increase the need for nutrient addition, especially during the establishment period. On the one hand, nitrogen fertilisation of SRC crops can lead to nitrate leaching (Dimitriou and Aronsson, 2004). On the other hand, some SRC species, especially *Salix,* have high nitrogen uptake capacity due to their rapid growth, offering the potential to remove nitrates from soil (Rowe, Street and Taylor, 2009). When combined with the removal of nutrients during coppicing, the high nutrient requirement can make them less efficient at nutrient cycling than other temperate forest (Ens, Farrell and Bélanger, 2013). Nevertheless, this high nitrogen requirement reduces nitrogen leaching and GHG emissions from soils under SRC plantations, especially *Salix* spp. (Ens, Farrell and Bélanger, 2013). Furthermore, established SRC crops may require less nitrogen input, as nutrient cycling becomes more efficient with plantation age (Ens, Farrell and Bélanger, 2013; Fabio and Smart, 2018; Ugilt et al., 2018; Agriculture and Horticulture Development Board, 2019).

Short rotation coppice can take up high levels of phosphorus, minimising leaching into nearby water bodies (Ens, Farrell and Bélanger, 2013). Plants are often unable to acquire phosphorus at the same rate it is returned to soil, creating a depletion zone in the rhizosphere; phosphate fertiliser is applied to many commercial crops to combat this (Da Ros et al., 2018b). Although the addition of phosphate does not lead to an increase in SRC crop yield, coppice trees such as *Populus* often store excess phosphorus in leaves, while some *Salix* varieties are able to store excess phosphorus in the tree stem (Da Ros et al., 2018b). Phosphorus stored in leaves is often returned to the soil during senescence, increasing soil phosphorus availability. Conversely any stored within stems is lost during coppicing (Da Ros et al., 2018b).

Growth of SRC crops under different conditions

If climate change continues to intensify as predicted, we can expect to see an increase in extremes of weather conditions. The definition of an extreme weather event is broadly defined as rare at a particular place and time of year but this is often subjective, with varying duration and intensity of dry or wet spells constituting extreme drought or flooding depending on location (IPCC, 2021). When considering drought or flood events precipitation quantity is most commonly used as a measure. The most recent studies focus on rainfall, which is either below the 5th percentile or above the 95th percentile of the daily mean rainfall for three days or more as the parameter for an extreme precipitation event (Dodd et al., 2021). Drought events can be broadly termed under two characteristics: i) duration, where the length of time with limited water availability creates drought conditions; and ii) intensity, where water limitation even for short periods creates drought conditions. Importantly, both drought and flood events can last days or months (Grillakis, 2019; Dodd et al., 2021).

Short rotation coppice crops grow well in marginal land such as wet or nutrient-depleted soils, offering land-owners the opportunity to improve soil conditions, biodiversity and revenue without compromising prime agricultural land for food production (Aylott et al., 2010). However, SRC yields rely heavily on water availability, as water use during warm summer months is much higher than other biomass crops (Aylott et al., 2008). *Salix* and *Populus* in particular have high water consumption rates during the warm growing season (Dimitriou et al., 2009). Thus, water deficit stress is a substantial concern for plant fitness in *Salix* and *Populus* SRC crops (Oliver, Finch and Taylor, 2009). The immediate impact of drought on plant fitness is obvious, as resources are diverted from plant organs such as leaves and focused on stem or root function (Mohamed et al., 2014) but drought also has significant impacts on morphological traits such as cell growth, leading to overall reduced crop yield (Jaleel et al., 2009). For example, although *Populus* SRC crops demonstrate short-term tolerance to moderate drought, crops subjected to longer or more severe drought have lower above- and below-ground biomass, which reduces bioenergy yield, plant fitness and ability to mitigate further environmental impacts (Shao et al., 2008). Drought therefore has significant implications for the energy value and economic benefits of SRC crops, although some studies have shown they can mitigate short-term drought by improving water use efficiency (Aylott et al., 2008).

Less is known about the legacy effects of drought on soil carbon dynamics and carbon storage in SRC crops. However, the impact of drought has been well studied in many other globally important crops, where drought has been shown to reduce plant available nutrients (Mingzhu and Dijkstra, 2014). Declines in basic plant functions such as photosynthesis and root exudation increase with prolonged drought periods (Ruehr et al., 2009), which can also reduce AMF colonisation (Compant, Van Der Heijden and Sessitsch, 2010a). Furthermore, intense drought periods are likely to reduce carbon allocation to woody biomass, reducing tree growth and affecting the carbon neutral status of the crop (Verlinden et al., 2013), while also promoting premature aging of the woody biomass (Larchevêque et al., 2011) and premature leaf drop (Köhler et al., 2020).

Increased precipitation immediately after a drought (i.e. rewetting) combined with increasing temperatures can increase plant biomass without altering soil respiration, indicating that drying–rewetting cycles could enhance carbon storage during rewetting periods (Templer and Reinmann, 2011). However, drying-rewetting cycles also alter soil properties such as nutrient availability and moisture holding capacity and often result in the release of substantial amounts of CO_2 to the atmosphere (Suseela et al., 2012).

Prolonged periods of drought can reduce crop yield, but soil GHG emissions may also be reduced. Although nitrogen addition can mitigate reductions in crop yield it also has the potential to substantially increase soil GHG emissions (Geng et al., 2017). By contrast, AM fungi can mitigate the effects of short-term drought on plants because they continue to facilitate the exchange of nutrients for carbon and can penetrate smaller pore spaces than roots are able to, which improves crop access to water in drying soils (Deepika and Kothamasi, 2015). Thus, fertilisation and AM fungal associations are likely to affect SRC crop resilience to drought events as well as soil carbon and nutrient dynamics. However, to my knowledge there are no studies that have investigated potential interactions between nutrient availability and AM fungi on crop yield or soil carbon dynamics upon rewetting after drought.

Softwoods like *Populus* and *Salix* are relatively tolerant to periodic flooding and are often found in periodically waterlogged soils in riparian habitats (Vreugdenhil, Kramer and Pelsma, 2006). Indeed, SRC crops could contribute to water management and flood mitigation (Rowe, Street and Taylor, 2009). Nonetheless, long-term waterlogging reduces nutrient uptake, limits growth and could enhance CH_4 emissions from the soil (Schindler et al., 2020a). Methane production can occur within days of waterlogging events and may take several weeks to stabilise (Le Mer and Roger, 2001; Krüger et al., 2005; Brzezińska et al., 2012). Methane production under SRC crops has received little attention but eddy covariance measurements suggest that CH_4 emissions during SRC crop establishment on marginal lands can partially offset carbon sequestration benefits (Verlinden et al., 2013). Importantly, extreme weather affecting soil water availability is rarely a single event such as drought or flood but rather an interaction of both (Gray and Brady, 2016). This combination of extreme events is referred to as ‘compound events’ (Zscheischler et al., 2020). For example, extended periods of drought conditions can be followed by extended periods of waterlogging (Dodd et al., 2021). As weather patterns move towards drier summers and wetter winters (IPCC, 2021), understanding how drought legacy interacts with intense periods of waterlogging is of great importance. Thus, to assess the carbon benefits of biomass crops under climate change we require an improved understanding of how changes in precipitation (particularly drought and flooding) and compound events will alter plant–soil carbon dynamics in SRC crops. Although breeding programs have improved SRC crop yield and drought tolerance (Oliver, Finch and Taylor, 2009), some modified hybrids have altered relationships with mycorrhizal fungi (Arraiano-Castilho et al., 2020; Lamit et al., 2021), which could affect their growth and nutrient uptake..

1.3 The potential importance of mycorrhizas for SRC crops

Mycorrhizal fungi play an essential role in many ecological processes, from soil fertility to cycling of organic matter and soil development; they are therefore considered vital in most ecosystems (Cumming et al., 2015; Finlay, 2008). Mycorrhizal associations are formed between fungi and plant roots. The fungi are heterotrophic, so they rely on their plant hosts to supply carbon. In return, the fungal partner produces hyphae that grow out from the roots into surrounding soil to forage for nutrients such as nitrogen and phosphorus, which often limit the growth of the host plants (Abbott and Robson, 1985). These associations between plants and mycorrhizal fungi are thus often mutually beneficial, and could be harnessed to improve SRC crop yield, abiotic stress responses or disease resistance, and reduce dependency on water and nutrient additions. Hence, understanding the potential benefits of mycorrhizal associations for SRC crops is important for developing and managing SRC crops that are resistant to unfavourable abiotic conditions.

Mycorrhizal symbiosis is considered the most ancient widespread form of fungal symbiosis with plants, dating back to initial colonisation of land (Smith and Read, 2008d). It is estimated that up to 90% of terrestrial plant species associate with mycorrhizal fungi, whereby arbuscular mycorrhizal (AM) fungi associate with around 80% of vascular plants and ectomycorrhizal (ECM) fungi form associations with around 10% of plants, most commonly top canopy woody species (Smith and Read, 2008d). While both AM and ECM fungi associate with roots, there are important differences in the way they interact with their plant hosts. AM fungi penetrate host root cell walls and develop arbuscules, which have a large surface area of contact with the plant cell membrane, forming the main site for the exchange of nutrients and carbon. By contrast, ECM fungi symbiosis occurs through a fungal mantle around young roots, where lignification has not yet begun. ECM fungi form three structures on and around roots to enable the exchange of nutrients and carbon: the mantle that encloses the root tip, the ‘Hartig net’ comprising hyphae that grow into the root between the epidermal and cortical cells, and the hyphal network that grows out of the root into the surrounding soil, known as extraradical mycelium (Smith & Read, 2008). Interestingly, the internal hyphae which form the Hartig net appear to grow across the root as opposed to along it, creating the contact zone between the root and fungi, while the mantle provides an area for nutrient storage (Smith & Read., 2008). The mantle is often connected to well-developed extraradical mycelia, which can extend over relatively large distances of many centimetres from the host plant, which increases foraging in the surrounding soil (Jörgensen et al., 2022). ECM have saprotrophic abilities and can stimulate the breakdown of dead organic matter, allowing young mycorrhiza the ability to obtain some C independently. However, this ability is lost over time where reliance on the supply of C from plants becomes vital (van der Heijden et al., 2015a). Conversely AM fungi have no saprotrophic ability relying solely on supply of C from plants (Smith and Read, 2008a). While both AM and ECM rely on extensive hyphal networks to forage for mineral nutrients that might otherwise be inaccessible to host plants, studies have shown that AM fungi are also able to utilise P supplied as rock phosphate (Finlay, 2008b). Thus, AM fungi have major implications for soil fertility in agriculture because they can greatly increase plant access to P.

The large body of research demonstrating the benefits of mycorrhizal fungi to plant nutrient acquisition and growth has led to the development of commercial AM fungal inoculum as an alternative to fertiliser (Igiehon and Babalola, 2017; Chen et al., 2018; Elliott et al., 2021). Using AM fungi to improve crop establishment and nutrition is attractive because the price of commercial AMF inoculum is low and the inoculation only involves the addition of AMF inoculum to the soil at the time of planting (Tauler and Baraza, 2015). SRC crops growing on degraded marginal land may benefit from increased nutrient acquisition through mycorrhizal associations (Pray et al., 2018) and commercial AM inoculation could facilitate their initial establishment and growth (Tauler and Baraza, 2015). Although the fungal partner exchanges the acquired nutrients in return for carbon (Read, Perez-Moreno and Perez-Moreno, 2003; Bender, Conen and Van der Heijden, 2015), the carbon cost to the plant is usually offset by enhanced nutrient acquisition, so understanding this balance could be important for determining the carbon sequestration capacity of SRC crops (Rooney et al., 2009).

Mycorrhizal fungi could enhance the carbon sequestration capacity of SRC crops by boosting productivity and promoting soil carbon storage (Rooney et al. 2009). Fungal hyphae are thought to increase soil carbon sequestration by promoting carbon allocation to belowground biomass (Rooney et al. 2009) and transporting carbon away from areas of high respiration and organic matter mineralization, e.g. around roots and decaying organic matter (Wilson et al., 2009). As hyphal networks can constitute large amounts of the total biomass in soils, significant amounts of carbon are transported though mycorrhizal mycelia, with estimates ranging from 15% to 28% of the total carbon uptake by photosynthesis (Finlay, 2008). Interrupting hyphal networks through girdling can reduce soil respiration by up to 43% (Brzostek et al., 2015), highlighting the importance of mycorrhizal hyphal networks in soil carbon dynamics. Mycorrhizal fungi also produce glomalin, a glycoprotein that is highly resistant to degradation and can persist in soils for decades (Wright and Upadhyaya, 1998). However, whereas ECM fungi are generally thought to enhance soil carbon storage, numerous studies demonstrate that AM fungi can also promote the turnover of soil organic matter and the release of carbon as CO_2

(Kuzyakov, 2010; Phillips et al., 2012; Cortrufo et al., 2013; Sulman et al., 2017). Indeed, ecosystems primarily associated with AM fungi tend to have lower soil carbon stocks than ecosystems primarily associated with ECM fungi (Averill, Turner and Finzi, 2014a). We still know relatively little about how mycorrhizal associations will affect soil carbon cycling in SRC plantations, but overall, the carbon allocated to fungal structures is thought to contribute to both short and long-term soil carbon pools (Rooney et al., 2009).

Mycorrhizas, fertilization, and abiotic conditions in SRC crops

How mycorrhizal fungi might interact with fertilisation of SRC crops is a primary area of interest for research (Rooney et al. 2009). Mycorrhizal fungi may alter plant nitrogen by increasing the amount of nitrogen available to the plant and increasing plant access to different forms of nitrogen (Hobbie and Högberg, 2012). Mycorrhizal fungi are able to take up nitrogen in the form of both nitrate and ammonium but they prefer to use ammonium as it is less energy intensive (Balestrini et al., 2020). Hence, soils with low availability of ammonium may be more susceptible to nitrogen depletion by mycorrhizal fungi (Hobbie and Högberg, 2012). Importantly, mycorrhizal communities often have a high nitrogen requirement and they will always fulfil their requirement before exchanging any with mutualistic partners (Treseder and Allen, 2002). Mycorrhizal fungi may thus help reduce nitrogen leaching and nitrous oxide emissions from soil due to storage of nitrogen in their hyphal network (Fang et al., 2020a), but they could also reduce nitrogen availability to plants (Ingraffia et al., 2020). Mycorrhizal associations with roots can also alter the mineralisation of carbon and nutrients by altering decomposition rates (Clemmensen et al., 2015). For example, deciduous trees associated with ECM fungi often produce leaf litter that decomposes at a slower rate than deciduous trees associated with AM fungi, resulting in distinct nitrogen and carbon cycling rates in systems dominated by ECM vs. AM fungi (Phillips, Brzostek and Midgley, 2013).

Although *Salix* and *Populus* can associate with both AM and ECM fungi (Dimitriou et al. 2009), we know very little about how interactions between fertilisation and mycorrhizal associations will affect carbon cycling and storage in biomass and soils in SRC crops. For example, the addition of nitrogen often increases aboveground biomass but this can occur at the expense of belowground carbon allocation, which could in turn reduce mycorrhizal fungi biomass and alter carbon and nutrient cycling (Högberg et al., 2010). Similarly, as plants generally have a higher phosphorus requirement than their mycorrhizal partners, phosphorus fertilisation can reduce plant carbon allocation to mycorrhizal fungi, impacting soil carbon cycling and potentially reducing carbon storage (Olsson, Rahm and Aliasgharzad, 2010). However, soils with high nutrient availability have microbial communities that are more likely to degrade fresh organic carbon inputs than soil organic matter (Fontaine et al., 2004; Zhou et al., 2014), which could boost soil carbon stocks (Fontaine et al., 2004a). Thus, there are substantial knowledge gaps around interactions between fertiliser application and mycorrhizal fungi, which could strongly influence soil carbon storage in SRC crops. Furthermore, shifts in abiotic conditions under climate changes will influence both mycorrhizal fungi and the fate of nutrient inputs from fertilizers, thereby affecting the productivity and carbon balance of SRC crops.

While there is a body of research into the benefits of SRC crops for bioenergy production, three vital areas have hitherto been overlooked: First, most analyses of carbon cycling and storage do not consider the potential impacts of changes in root biomass and microbial activity on the carbon neutral status of SRC crops. Second, when considering the establishment of bioenergy crops on marginal or degraded land, few studies have assessed how increased fertiliser use might affect soil gas fluxes, and how soil GHG emissions could be affected by adverse abiotic conditions such as drought or flooding. Finally, very few studies have investigated mycorrhizal inoculation to aid the establishment of SRC crops or individual extreme weather conditions, and none have assessed how mycorrhizal associations might benefit crop yield and soil functions during compound extreme weather events.

1.4 Thesis objectives

The overarching objective of this thesis is to increase our understanding of how soil fertility and mycorrhizal associations influence carbon cycling and storage in SRC crops and how these interactions are altered by abiotic stress (drought and flooding). Closing knowledge gaps around the interactions between soil fertility, mycorrhizal associations and abiotic stress is important because SRC crops are often grown on marginal land with infertile soils or highly fluctuating water avaliablity. The body of work presented in this thesis focuses on willow (*Salix sp.*) and poplar (*Populus sp.*), as they are the most commonly used species for SRC crops in the UK and are recommended as biomass crops by the UK government. Specifically, my research aims to:

1) Establish how nutrient addition, mycorrhizal inoculation, and their interactions impact upon plant fitness and soil respiration.
2) Establish how nutrient addition, mycorrhizal inoculation, and their interactions impact upon soil respiration and nutrient availability under two SRC crop species after an intense drought – rewetting event.
3) Establish how nutrient addition affects soil GHG emissions under two SRC crop species during an intense flood event.

The body of work presented in my thesis advances the field by quantifying changes in tree growth and soil carbon dynamics, which will inform large-scale field studies to compile whole life cycle carbon assessments for SRC crops. By discussing differences in the responses of the two species, I provide valuable information on their potential suitability under different scenarios.

Thesis outline

This thesis comprises five chapters: the present introduction (Chapter 1) provides an overview of the subject area and the specific topics of interest; Chapters 2, 3, and 4 present empirical experiments focusing on each of the research aims in Section 1.5.

The experiment in ***Chapter 2*** forms the foundations of the thesis by assesing the impacts of nutrient addition, AM fungal inoculation, and their interactions on plant growth and soil respiration (CO_2 efflux). As both fertilisation and AM fungal inoculation have the potential to influence plant growth, C storage and soil C dynamics, the study presented in Chapter 2 tests the following overarching hypothesis:

- Nitrogen addition and AMF inoculation will increase carbon storage in plant biomass, but AMF inoculation will also boost soil CO_2 efflux.

The experiment in ***Chapter 3*** focuses on investigating how mycorrhizal inoculation or fertilisation influence carbon and nutrient dynamics upon rewetting after drought. As fertilisation with nitrogen and inoculation with AM fungi influence plant responses to drought, and subsequent rewetting has profound impacts on soil carbon and nutrient dynamics, the experiment tests the following overarching hypotheses:

- Nitrogen addition and AMF inoculation will reduce the impacts of drought and rewetting on soil CO_2 efflux and nutrient availability.

Chapter 4 builds on this to investigate how coupound events (drought and flooding) interact with nitrogen fertilisation to influence soil CO_2 and CH_4 emissions. Here, I tested the overarching hypothesis that:

- Flooding will reduce tree growth and increase soil CO_2 and CH_4 emissions under SRC crops, but soil CO_2 and CH_4 emissions will be lower under trees with a history of drought.
- Nitrogen addition will offset the negative effects of flooding on tree growth but enhance soil CO_2 and CH_4 emissions; the effects of nitrogen addition will be lower under trees with a history of drought.

Finally, ***Chapter 5*** discusses the findings of the research in the context of current 2050 Net Zero targets. and highlights emerging research questions.

To address the aims of this thesis, I carried out three experiments using pot-grown trees under controlled watering and nutrient addition regimes during three growing seasons. All experiments described in this body of work were carried out using the general set up described in Chapter 2.

2. Growth and carbon dynamics of willow & poplar under nutrient amendment and mycorrhizal inoculation

2.1 Introduction

Globally, the upper 100 cm of soils are estimated to contain around 1500-Pg of organic carbon (Hawkes et al., 2008) and soil organic matter (SOM) contains over three times as much carbon (C) than the atmosphere or terrestrial vegetation (Schmidt et al., 2011). In agricultural systems, soils are being lost at a rate of c. 10 million ha per year due to degradation, which is accelerating losses of SOM and reducing soil C stocks (Kopittke et al., 2017). Changes in land use can increase atmospheric CO_2 contributions from managed land through fertilizer application and by altering the soil structure and abiotic conditions, which increase soil microbial activity and release CO_2 (Buckingham, Rees and Watson, 2014). Consequently, agriculture and forestry combined are the second largest cause of greenhouse gas emissions globally, partly due to increased fertilizer use and conversion of degraded agricultural land for urban development (Lal, 2004). However, degraded and marginal soils are also increasingly being used to grow bioenergy crops, especially woody perennials such as *Populus* (poplar) and *Salix* (willow) spp. because they are fast-growing, have a high bioenergy content and grow well in poor soil conditions (Edrisi and Abhilash, 2016). Such woody perennial crops can help reduce or offset agricultural CO_2 emissions because they are grown as short rotation coppice (SRC), in which the crops grow for up to 20 years and are usually only cut every three years (Aylott et al., 2008; 2010; Wickham et al., 2010). Despite considerable variation among crops grown under different climatic and soil conditions (Hangs et al., 2014), SRC bioenergy crops may produce significantly fewer greenhouse gas emissions than other agricultural crops (Hillier et al., 2009). However, increasing use of marginal and degraded land may increase the frequency and intensity in which fertiliser additions must be applied to achieve optimum yield. Current Department of Environment Food and Rural Affairs (DEFRA) guidelines recommend that no fertiliser should be added during SRC establishment, followed by increasing amounts over the subsequent three years, but accurate fertiliser requirements of short rotation crops are still unknown (DEFRA, 2004). Increasing fertilisation could stimulate microbial activity in the soil by providing nutrients to boost growth and enhancing plant inputs, increasing heterotrophic respiration and thus CO_2 emissions from the soil (Moscatelli et al., 2008; Schweier et al., 2017; Nguyen et al., 2018b). The release of CO_2 from the soil is exacerbated by blanket application of fertilisers, because only *c*. 40-50% of the added nitrogen (N) and 45% of the added phosphorus (P) is taken up by plants (Kopittke et al., 2017). In addition, changing environmental conditions are altering plant–soil interactions, which could affect soil C sequestration and CO_2 emissions under bioenergy crops (Oliver, Finch and Taylor, 2009). Consequently, to fully assess the C benefits of bioenergy crops, we first need to improve our understanding of how fertiliser additions and a changing climate influence plant–soil interactions and CO_2 emissions from the soil (Buckingham, Rees and Watson, 2014).

Understanding how nutrient availability influences plant-soil C dynamics in bioenergy crops is important because fertiliser management is not only critical for ensuring productivity, but it can also play a key role in soil C sequestration (Rytter, 2012; Edrisi and Abhilash, 2016; Georgiadis et al., 2017; Fabio and Smart, 2018). A recent review demonstrated that soil C turnover is influenced by biome type, abiotic conditions, climate changes, and N deposition, as well as the intensity and duration of fertiliser additions (Zhou et al., 2014). Hence, improved management of fertiliser use in agricultural systems can contribute to stabilising SOM and soil C stocks (Kopittke et al., 2017). Nitrogen and P are applied widely to bioenergy crops because they are the key elements for plant growth (Moscatelli et al., 2008; Hangs, 2013; Agriculture and Horticulture Development Board, 2017; Georgiadis et al., 2017). Nitrogen is an essential element in amino acid and protein synthesis and chlorophyll development; it promotes growth, encourages early root growth, and facilitates the uptake and exploitation of other essential nutrients such as potassium (K) and P (Leghari et al., 2016). Nitrogen limitation is insidious in terrestrial ecosystems, limiting transfer of C in both plants and soil organisms (Högberg et al., 2010). Phosphorus is required for enzyme production and energy metabolism, and thus P limitation precipitates physical and hormonal stresses encouraging adaptations to overcome these issues (Cumming et al., 2015). When plant nutrient demand exceeds the availability of soluble nutrients in the soil solution, plant inputs to the soil can promote the activity of soil microbial communities capable of mining SOM to release nutrients. This so-called ‘priming’ of SOM was first discovered by Löhnis (1926), who showed that inputs of fresh plant-derived C to the soil can accelerate the mineralisation of SOM, releasing soil C as CO_2 and making the nutrients stored in SOM (primarily N) available for plant uptake. However, negative priming can also occur when nutrient levels in soil solution are high (Kuzyakov, Friedel and Stahr, 2000), which reduces mining of SOM by soil microbial communities and increases soil nutrient and C storage (Fontaine, Mariotti and Abbadie, 2003; Fontaine et al., 2004b; 2011). Thus, the stability of soil C stocks is affected by nutrient availability, and the priming effect can have an impact upon both C and N sequestration in soils (Bradford, Fierer and Reynolds, 2008). Numerous studies have demonstrated that N-addition can increase the storage of SOM in forest ecosystems (Berg and Matzner, 1997; Bowden et al., 2019; Wang et al., 2019), but it is unclear whether N fertilization of SRC bioenergy crops will have the same effect. Indeed, despite several recent studies focusing on the C sequestration benefits of SRC (Lockwell, Guidi and Labrecque, 2012; Verlinden et al., 2013; Quinkenstein and Jochheim, 2016), few have considered how nutrient status of these crops influences soil C turnover and CO_2 emissions.

The ability of perennial bioenergy crops such as poplar and willow to form a mutualistic relationship with mycorrhizal fungi could reduce the need for fertilization and contribute to soil C storage (Fillion et al., 2011; Hrynkiewicz et al., 2012; Liu et al., 2015). Both poplar and willow species associate with arbuscular mycorrhizal (AM) fungi and ectomycorrhizal (ECM) fungi (Munro and Atkinson, 1992; Dhillion, 1994; Trowbridge and Jumpponen, 2004; Fillion et al., 2011; Becklin, Pallo and Galen, 2012; Hassan et al., 2014; Liu et al., 2015; Weih, Glynn and Baum, 2019). The fungal partners provide nutrients to host plants in return for C in the form of sugars (Bender, Conen and Van der Heijden, 2015; Read and Perez-Moreno, 2003). The mycorrhizal fungal partners play an important role in host plant nutrition because the fungal hyphae extend much further into the soil than plant roots and thus outperform roots in foraging for nutrients (Smith et al., 2010). The benefits of this mutual exchange of C and nutrients between host plants and mycorrhizal partners have led to the development of commercial mycorrhizal inoculants to stimulate plant growth and reduce the need for regular fertilization in horticultural plants and agricultural crops (Cozzolino, Di Meo and Piccolo, 2013; Faye et al., 2013; Poeplau, 2021). However, the extent of the hyphal network and the efficiency with which the mycorrhizal fungi can obtain, transport and exchange nutrients depends strongly on both the plant and fungal species (Lee et al., 2013) and the success of mycorrhizal inoculation of commercial crops can be highly site-specific (Kokkoris et al., 2019). It is also important to note that the benefits of mycorrhizal associations to host plants can be strongly influenced by nutrient availability, especially N (Treseder and Allen, 2002; Treseder, 2004; Parniske, 2008; Hodge, Helgason and Fitter, 2010; Van Der Heijden, 2010; Kivlin, Hawkes and Treseder, 2011a; Antunes et al., 2012; Vieterhausen, 2013). As mycorrhizal fungi have a high N requirement, they will not transfer N to the host plants until their own requirements are fulfilled (Johnson et al., 2010). By contrast, fungal P-requirement is generally lower than plants (Etesami et al., 2021) but fungal mycelia are able to access plant inaccessible P by foraging in areas outside of the immediate root zone, allowing uptake in excess of plant needs and exchange P with plant-derived C in the root arbuscule (Schachtman, Reid and Ayling, 1998). Taken together, this suggests that mycorrhizal fungi will transfer P much more readily than N to host plants in exchange for C (Finlay, 2008a).

Mycorrhizal associations also appear to be strongly linked to soil C storage and soil CO_2 emissions (Averill, Turner and Finzi, 2014b), which is at least partly explained by differences in nutrient availability and the relative nutrient demands of host plants and fungal partners (Augé, 2001; Wilson et al., 2009; Näsholm et al., 2013; Ven et al., 2019). Importantly, AM fungi have high rates of mycelial turnover, which provides C inputs to the soil (Staddon et al., 2003) but several studies suggest that AM fungi can stimulate the mineralisation of SOM and the release of C as CO_2 (Paterson et al., 2016; Tedersoo and Bahram, 2019). However, given that plant-mycorrhizal mutualism is based on the provision of C by plants to the fungal network in return for enhanced nutrient uptake, the propensity of mycorrhizal fungi to store or release C is also likely to be influenced by soil fertility (Wilson et al., 2009; Phillips, Brzostek and Midgley, 2013). Hence, although mycorrhizal associations could reduce the need for fertilizer application in bioenergy crops, interactions between roots, mycorrhizas and other associated soil organisms can significantly influence soil C and nutrient dynamics (Talbot, Allison and Treseder, 2008). Consequently, it is unclear how mycorrhizal status and fertilizer management will influence soil C dynamics and CO_2 emissions in perennial bioenergy crops.

In this study, I aimed to assess the relative benefits of fertilization and mycorrhizal inoculation on the establishment and early growth of the perennial bioenergy crop species poplar and willow, while assessing how combinations of N-addition, P-addition and mycorrhizal inoculation influence soil CO_2 emissions. I grew poplar and willow saplings in mesocosms with added N-fertiliser, P-fertiliser, and a commercial mycorrhizal inoculum. I measured plant growth rates, biomass, soil CO_2 efflux and soil properties to test the following hypotheses:

H1. Addition of N will increase plant biomass and C storage within the woody stem, whereas P addition will have a limited effect on woody biomass but will increase leaf biomass.

H2. AMF inoculation will increase plant biomass and reduce the need for N and P addition. Carbon storage in plant biomass will increase with AMF inoculation, and soil respiration will also increase as the plant exchanges C in return for nutrients.

H3. The addition of N will reduce total soil respiration as nitrogen mining within soil is reduced, whereas P-addition and AMF inoculation will have the opposite effect.

2.2 Materials and methods

Experimental design and setup

To establish how fertiliser and mycorrhizal inoculation influence bioenergy crop yield and plant–soil C dynamics, I used a large pot study with two tree species that are commonly planted as bioenergy crops within the UK: *Populus nigra* L. (poplar) and *Salix purpurea* L. (willow) grown under four fertilizer and two mycorrhizal treatments in a fully factorial design. The four nutrient treatments were: nitrogen addition (+N), phosphorus addition (+P), both nutrients (+NP) and a control treatment with no added nutrients (CT). The soil in each pot was either inoculated with arbuscular mycorrhizal fungi (+AMF) or not (-AMF). There were six replicate blocks, each containing two sets of pots per treatment combination and tree species, giving a total of 192 trees.

In April 2018 I set up a trial study to establish effective methods for inoculating poplar and willow cuttings with ectomycorrhizal fungi (ECM). I grew poplar and willow trees from 25cm cuttings in a 1:1 sterile loam and sharp sand mix in polytunnels at The James Hutton Institute, Dundee, Scotland. I chose five different species/strains of ectomycorrhizal fungi *Laccaria bicolor* s238n, *Laccaria bicolor* 12, *Laccaria laciata* sk33, *Laccaria bicolor* n203 and *Hebeloma crustuliforme* up184. These species are pioneer species and, with the exception of *H.crustuliforme,* up184 are considered aggressive colonisers. Each species was grown on sterile plates with modified melim morkrans medium (MMN), sealed using parafilm M tape, incubated at 20°C and checked monthly for growth. In total, 10 plates per species were grown. To inoculate the trees, the 10 plates of inoculum per species were placed in a blender goblet adding 500 ml distilled water and blended on low. This step was repeated for each of the five isolates, which were then combined into a single mixed ECM inoculum. The volume of the resulting ECM slurry was increased to 5 L with distilled water, to ensure the solution was fully mixed. The trees were inoculated in May 2018 . The poplar and willow root balls were gently cleaned of soil to expose roots. Each cutting was then placed into a pot with the sterile 1:1 loam and sand mix and covered with 25ml of ECM slurry, ensuring all roots were coated.

At the end of the growth season in October 2018 trees were harvested to establish colonisation. For each tree the roots were washed and sampled for mycorrhizal colonisation. A sub-sample of root tips was collected, placed into 10-ml test tube, and covered with ethanol, ensuring that only young white roots where collected. The root tips were then placed into a petri-dish, covered with deionised water and assessed for colonisation under a dissecting microscope (6×, 12×, 25×), using a black background and lamps of daylight quality. At this stage, there was no evidence of ECM colonisation. ECM inoculated roots often have short lateral roots coming off main roots, these tend to be thicker and identifiable due to the mantel hyphae and Hartig net. ECM inoculated roots will often have clear mycorrhizal hyphae spreading from the colonised root tips into the soil (Smith and Read, 2008). I found no evidence of any of these indicators of colonisation. Given the lack of evidence for colonisation, and the lack of literature to suggest that artificial inoculation of trees with ECM was achievable in a pot study, I decided to drop ECM from the study going forward.

In May 2019, I set up the study used for this thesis. I set up 192 18-L pots (30-cm diameter, 30-cm height) in two commercial polytunnels with ends covered with mesh to allow air circulation, at the James Hutton Institute, Dundee, Scotland. Both polytunnels were oriented from east to west and had two benches running along the length, with c. 2-m space between benches. The benches were 1-m wide, constructed of two breeze blocks and a wire mesh laid on top at c. 88-cm above ground level. Each bench held 48 pots (18-l, 30-cm x 30-cm) placed in a zigzag pattern; pots were sterilised in 1% bleach solution for four hours and rinsed before use (Figure 2.1a). Each polytunnel was split into three blocks per species (six replicate blocks in total), and 16 pots were assigned to each block (Figure 2.1b).

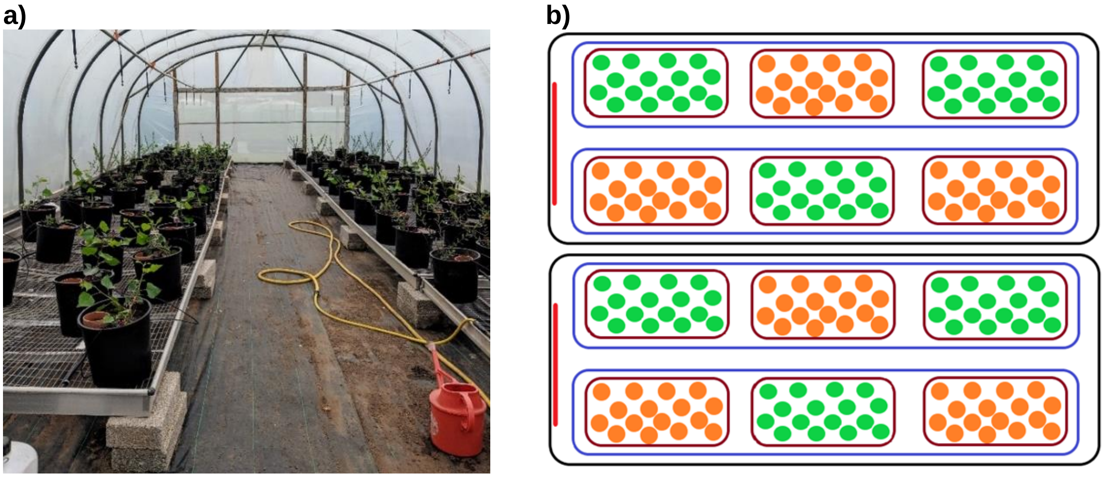


Figure 2.1 a) *A fully set up poly tunnel with pots in place and* ***b)*** *Diagram of the polytunnel set up. Each polytunnel had two benches (purple rectangles) with three blocks per species (orange or green circles in brown rectangles); treatments within a block were fully randomised. The polytunnels had a West to East orientation, with doors situated on the West-facing aspect, as indicated by the red line.*

All pots were filled with 17.5-L sterilised loam (Keith Singletons, Cumbria, UK) in a 1:1 mix with sharp sand. A commercial loam was used to standardise experimental conditions and reduce the potential confounding influence of variability in soil nutrients, organic matter, or microbial communities. Once mixed with sand, the soil was autoclaved for two hours and left to cool overnight. Initial analysis of soil properties was carried out on the sterilised soil mixture 30 days after autoclaving, to allow for nutrient levels to normalise after autoclaving. Ten random samples of soil were collected and homogenised and then analysed for extractable P and inorganic N (see section *Nutrient analysis*). Baseline soil nutrient concentrations post sterilisation were 72.87 mg N g^{-1} and 31.13 ug P g^{-1}.

To ensure the same volume of soil was added to each pot, I used 5-L and 2.5-L scoops; each scoop was tapped twice and levelled before the soil was added to the pot. Half of the pots were left as uninoculated controls (-AMF) and half of the pots per block were inoculated with a commercial AM fungal inoculant (+AMF). In total, I mixed 500-L of sterilised soil with 5-kg of RootGrow^{TM} Professional granules (PlantWorks Ltd, Kent, UK) in batches using a sterile cement mixer (Figure 2.2). I then added the soil to each pot in three layers: 5-L of sterilised soil, followed by 7.5-L of soil mixed with inoculum, ensuring an even spread of inoculated soil through the area in which roots will establish first, then a final layer of 5-L sterile soil. The inoculum is described as providing 1.6 million total propagules per litre supplied in a granule medium with zeolite particles. The inoculum mix comprises of *Funneliformis mosseae, F. geosporus, Claroideglomus claroideum, Rhizophagus irregularis* and *Glomus microagregatum.* PlantWorks guarantee that no fertiliser of any kind is added to the mix (Austen et al., 2022; Thirkell et al., 2022) .

Figure 2.2. *The sterile cement mixer used to ensure AMF inoculated soil was fully homogenised.*

I grew poplar (The Poplar Tree Company, Madley, UK) and willow (The Willowbank, Lydbrook, UK) from 30-cm cuttings. To establish the cuttings, I sterilised 200 Deepots^{TM} (D25L, 26-cm depth, Stuewe & Sons, Oregon, USA) in 1 % bleach solution for four hours. All pots where then rinsed in cold water to remove the bleach solution and lined with a layer of paper towel to cover the drainage holes and reduce soil loss. Each pot was filled with 650 ml of sterilised loam mix and then planted with one cutting of either willow or poplar, inserted 25-cm into soil (Figure 2.3a). All pots were hand-watered daily for four weeks before repotting. After four weeks of growth and root establishment, 96 cuttings per species were transferred to 18-L pots (16 pots per species and replicate block) in May 2019. First, I made a *c.* 25-cm deep hole to the centre left of each 18L pot using a sterile Deepot^{TM}. I then removed the cuttings individually from each Deepot^{TM}, gently rinsed the roots with cold water and placed the cutting root first into the hole in the 18L pot. Half the cuttings were planted in the +AMF pots, half in the -AMF pots and all cuttings were trimmed so that only the main branch was left. Once all cuttings were re-potted, the pots were watered and the cuttings left to establish for 30 days, allowing AMF inoculation to occur before applying fertiliser (Figure 2.3b).

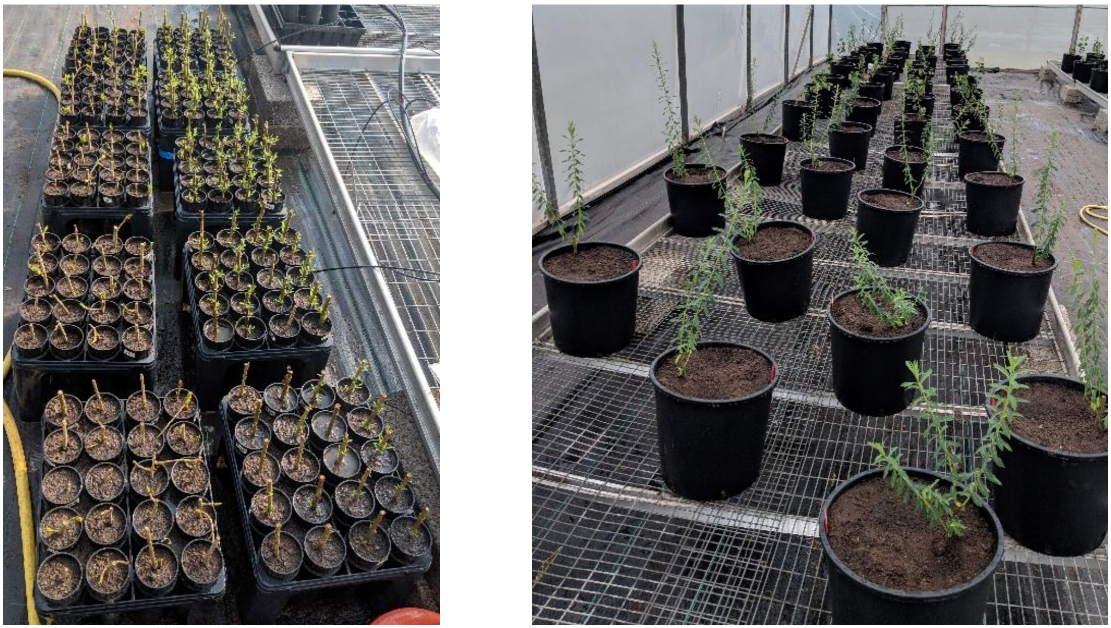


Figure 2.3 a) *Photo of the 30-cm cuttings of willow and poplar planted in 25-cm Deepots^{TM}, and* ***b)*** *the cuttings re-potted at four weeks into 18L pots.*

I applied one of four fertilizer treatments to 48 pots (six pots per species and mycorrhizal treatment): added nitrogen (+N), added phosphorus (+P), both nutrients added (+NP) or unfertilized controls (CT). Pots assigned to the +N treatment received 8.46-g KNO_3 and 2.2-g NH_4NO_3, pots assigned to the +P treatment received 7.3-g K_2PHO_4, and pots assigned to the +NP treatment received 2.2-g NH_4NO_3 and 7.3-g K_2PHO_4. The fertilizers for each treatment were dissolved in 100-ml deionised water and unfertilized controls received 100-ml deionised water. Thus, the fertilizer treatments represented rates of 120-mg N L^{-1} yr^{-1} and/or 80-mg P L^{-1} yr^{-1} and 202-mg K L^{-1} yr^{-1}, which is equivalent to high fertilization levels in commercially grown bioenergy crops (Aronsson, Bergström and Elowson, 2000; Weih, 2001; Agriculture and Horticulture Development Board, 2017; Nordborg et al., 2018). Thus, nitrogen concentrations were 193 mg N g^{-1} in N-fertilised soils compared to 72.87 mg N g^{-1} in control soils, and phosphorus concentrations were 111 mg P g^{-1} in P-fertilised soils compared to 31.13 ug P g^{-1} in controls soils.

Each pot was watered automatically at 8.30 am and 3.30 pm for 90 seconds, using two pressure-compensated spray stakes (flow rate 0.2 L min^{-1}; Netafim UK Ltd. Skelmersdale) fed by onsite mains water supply. Spray stakes were located on opposite sides of each pot, with the spray directed towards the centre (Figure 2.4a).

To measure soil CO_2 efflux, a permanent soil collar was installed in each pot. The collars were constructed of 110-mm drainage pipe inserted c. 10-cm into the soil, leaving 2-cm above the soil surface (Floplast Ltd, Kent, UK). All collars were sterilised in 1% bleach solution for four hours and rinsed thoroughly and then installed on the opposite side to the tree in each pot, taking care to minimise soil disturbance and root damage (figure 2.4a).

Figure 2.4 a) *Tree in place with irrigation stakes providing automated watering, showing the soil moisture probe and a 110 mm plastic drainage pipe providing an anchor for in-situ gas analysis, and* ***b)*** *the 10-cm chamber attached to an infrared gas analyser used to perform monthly soil respiration measurements.*

Monthly measurements

All monthly measurements were made during two growing seasons: from 3 June 2019 to 21 October 2019 and from 30 March 2020 to 24 August 2020.

Soil water content was measured monthly using a soil moisture probe (PMS-714, Lutron, Taipei, Taiwan), which was inserted into the soil to 10-cm depth every four weeks. The probe was sterilised with ethanol between pots to limit cross-contamination between +AMF and -AMF pots (Figure 2.4a).

To determine how fertilization and mycorrhizal inoculation influenced productivity, I measured tree growth every four weeks, I measured the basal diameter for each tree at the soil surface using digital stainless-steel Vernier callipers (J-Bonest, UK). I measured the height of the main stem on each tree using the starting point of new stem growth from the cutting as the base, using a 30-m fibron measuring tape (Rabone Chesterman, Birmingham, UK).

To assess the effects of fertilization and mycorrhizal inoculation on soil carbon dynamics, I measured soil respiration (CO_2 efflux) every four weeks using an automated soil gas flux system (Li-8100A; LiCOR Biosciences, Lincoln, Nebraska, USA) with a 10-cm diameter survey chamber. Each measurement lasted 2 mins with a 15-s pre-purge period and a 30-s dead-band. All data were downloaded and processed using the SoilFluxPRO software (LiCOR Biosciences, Lincoln, Nebraska, USA; Figure 2.4b).

Biomass harvest

To establish the success of fungal colonisation and provide an initial assessment of treatment effects belowground, I destructively harvested a subset of trees (two pots per species and treatment) after six months of growth, once all leaves had senesced at the end of the growing season in November 2019. To quantify above-ground biomass, I cut the trees at the soil interface, separated leaves from branches and stems, and dried leaves and woody biomass. The woody biomass was chopped into *c*. 50-cm sections, and oven dried at 70°C for 7 days before measuring dry weight. The leaves from each harvested tree were collected, dried to constant weight at 70°C, and weighed. Root biomass was determined once above-ground biomass had been harvested and soil samples taken. The remaining soil and roots in each pot were removed from the pot and the root ball was gently separated from the soil. The remaining soil was sieved through a 10-mm sieve and then through a 5-mm sieve (Endecotts Ltd, London, UK). The main root ball was soaked to remove excess soil. The roots were then gently washed over nested 10-mm and 5-mm mesh sieves to remove any remaining soil. Roots were separated from the main root stem and below-ground biomass was dried at 70°C for 7 days to determine dry weight (Figure 2.5).

Figure 2.5 *Soil wash bay during root washing, showing the root ball removed from soil, the sieves used and the remaining soil ready to be wet sieved.*

Nutrient analysis

To determine extractable P concentrations in the soil, I carried out Olsen's P extractions. Briefly, 2-g of air-dried soil and 10 ml of 0.5 M $NaHCO_3$ solution were added to a 50-ml tube. The tubes were shaken for 1 hour, centrifuged at 450 rpm for 5 minutes, and then 2-ml of supernatant was transferred to Eppendorf tubes. The P concentrations in the extracts were analysed on a spectrophotometer using the malachite green method. In brief, 15 µl of each sample was pipetted into a 96-well plate, using a clean pipette tip for each sample, and 185 µl of deionised water was added to each well. One row per plate was used to construct a calibration, whereby each well received aliquots between 0 and 80-µl of phosphate solution (2 µg P mL^{-1}) and deionised water was added to make the total volume 185-µl per well. All wells then received 100 µl of Malachite Green reagent. The plates were incubated at room temperature for 45 min and read at 620 nm absorbance using a Multiskan GO plate reader (Thermo Scientific, Waltham, MA, USA). Phosphorus concentrations were determined relative to the calibration curve and expressed as soil dry weight (Olsen & Sommers, 1982).

To determine soil inorganic N, I carried out KCl extractions. Briefly, I added 10-g of fresh soil and 40-ml of 1M KCl to a 50-ml glass jar. The jars were shaken for 45 minutes at 100 rpm and then filtered (Whatman 42 filter paper). Blanks with extraction solution only were also shaken and filtered. The extracts were stored at -20°C (Maynard et.

al.,1993) until analysed using a Seal autoanalyzer 3 (SEAL Analytical Ltd, Wrexham, UK).

To determine soil carbon and nitrogen and plant allocation of carbon and nitrogen to leaves, a subsample of each dried sample of leaves and soil were ground to a fine powder using a ball-mill (Mixer Mill MM 200, Verder Scientific Ltd, Hope, UK). Milled samples were analysed for carbon, nitrogen via combustion (Vario EL Cube, Elementar Ltd, Stockport, UK) and C:N ratio was calculated from the obtained values.

AM fungal colonisation

To determine AMF colonisation, dried root samples were placed into tissue cassettes, cleared in 3% KOH at 102ºC for 25 minutes, acidified in 2% HCI for 30 minutes, and stained with 0.05% Trypan Blue for 30 minutes. The tissue cassettes were then stored in de-stain solution and refrigerated until analysis of root colonisation. Roots were removed from cassettes and placed on microscope slides with a minimum of ten roots per slide where possible. Slides were left to air dry for five days and then polyvinyl lactoglycerol (PVLG) was added to affix cover slips (Figure 2.6). I then assessed the proportion of root length colonised by AM fungal hyphae, arbuscules, vesicles and spores using a compound microscope and the gridline intersect method (McGonigle et al., 1990). Briefly 100 root fragments were assessed per slide by moving through the slide and assessing presence or absence of mycorrhizal structures in each fragment.

Figure 2.6 *Root fragments that have been dried, cleared, stained, and placed on slides ready for root scoring to assess AMF colonisation.*

Data analysis

All analyses were performed using R version 4.1.1 (R Core Team, 2021), using the lme4 *(*Bates et al. 2015), MuMIn (Barton, 2020), lmerTest (Kuznetsova, Brockhoff, Christensen, 2017) packages for linear mixed effects models. All results were graphed using the ggplot2 package (Wickham and Chang, 2016). Data were transformed where necessary to meet modelling assumptions. The fertilization treatments were analysed factorially, whereby +N and NP treatments are “N-addition” and +P and NP treatments are “P-addition”. Relative growth rates for tree stem height and diameter were calculated by dividing the final values for stem height or diameter by the initial values.

The effects of fertilisation and AMF inoculation on soil extractable P, inorganic N, total C, total N were assessed using linear models (*lm* function) for each tree species separately. Explanatory factors were N and P fertilisation (four levels), AM fungi inoculation (three levels) and their interactions. Block was used as an error term. The effects of fertilisation and AMF inoculation on tree diameter and height growth (relative growth rates) were assessed using linear models (*lm* function) for each species separately, fitting factorial N and P fertilisation, AMF inoculation, and their interaction as explanatory variables, and block was included as an error term. The effects of fertilisation, AMF inoculation, and their interactions on leaf, wood, and root biomass were also assessed using linear models, but as only two pots per species and treatment were destructively harvested, both species were included in a single model, with species as the error term. Models were simplified by sequential exclusion of terms until the minimum adequate model was identified (Crawley, 2015; Appendix A, Table A1).

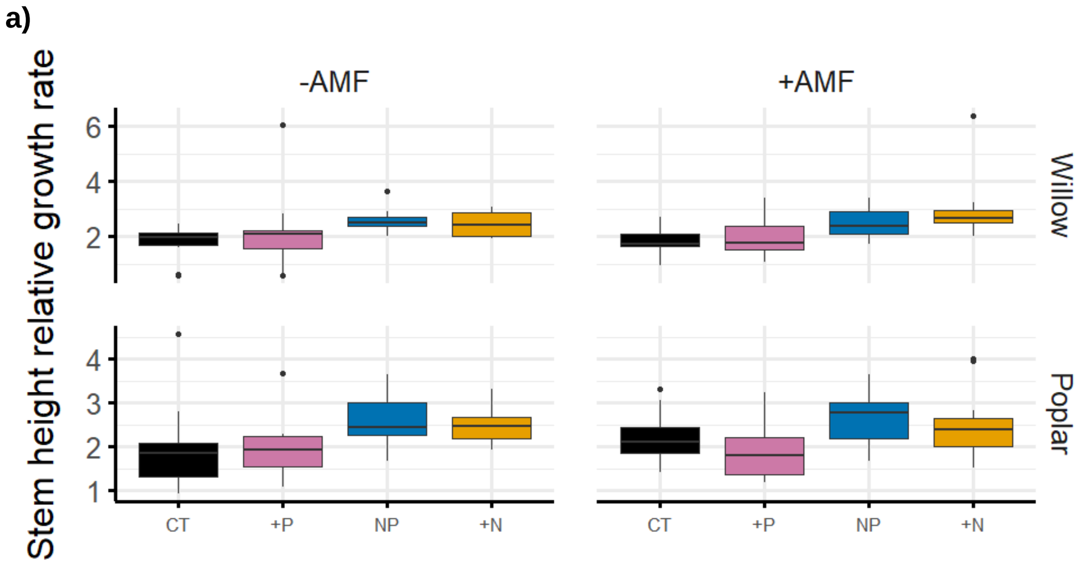
The effects of fertilisation and AMF inoculation on monthly soil respiration were assessed using linear mixed effects models (LMEs). The full model included N and P fertilisation, AMF inoculation and their interactions as fixed effects; to account for repeated measures data, block and time were fitted as random effects. The best model fit was then determined by sequentially dropping non-significant terms using the *dredge* function in the MuMIn package (Barton 2020). The final model was tested against a corresponding null model using a likelihood ratio test and the fit of the final model was checked using diagnostic plots. All results are reported as significant at $p < 0.05$ and as marginally significant trends at $p < 0.1$. For LMEs, the χ^2 and p-values from the likelihood ratio test are given for the best fit model, and the significance of individual terms was determined using Satterthwaite’s approximation to estimate degrees of freedom. Full model statistics and fixed effects coefficients are given in Appendix A, Table A2.

2.3 Results

Surprisingly, none of the root samples of either species showed colonisation by AM fungi. Nonetheless, inoculation with AMF affected tree biomass and soil respiration rates in at least one of the two species. However, N addition had by far the greatest effect on tree growth, biomass, and soil respiration.

Tree growth and biomass

Fertilization with P had no effect on tree growth, but stem diameter growth of both species increased significantly by 34% and 22%, respectively, with N-addition (poplar: $F_{4,92} = 9.8$, $p < 0.001$; willow: $F_{4,93} = 22.5$, $p < 0.001$). Height growth in willow was not influenced by fertilization, but height growth of poplar increased by 39% with N-addition ($F_{2,88} = 8.4$, $p < 0.001$). Inoculation with AMF had no influence on stem or diameter growth in either species (Figure 2.7; Appendix A1).



b)

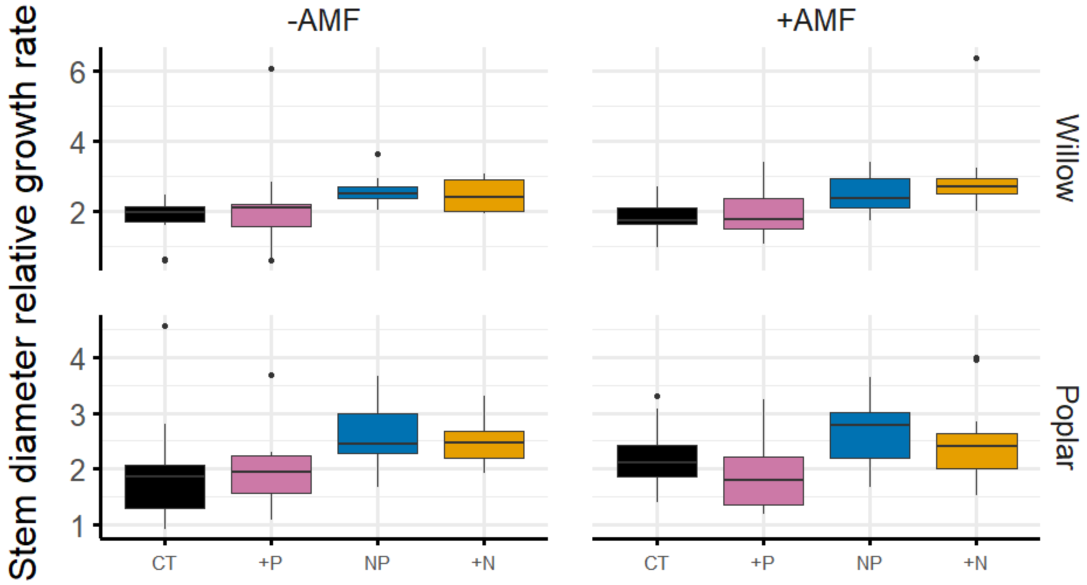
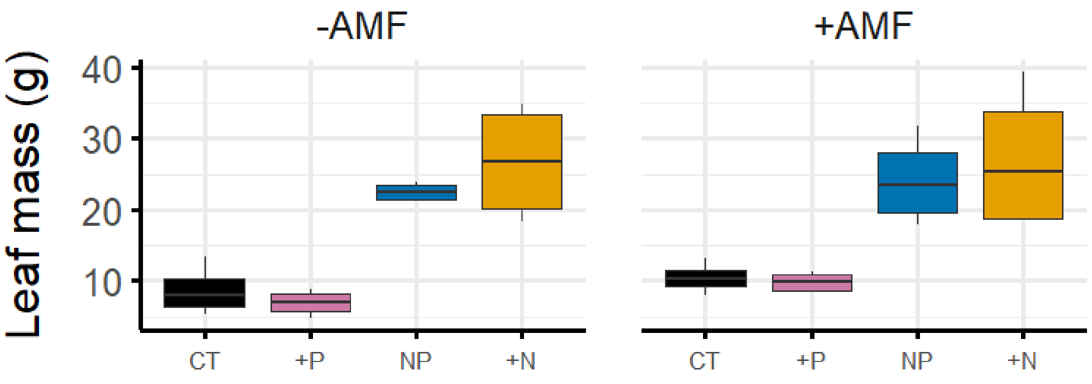


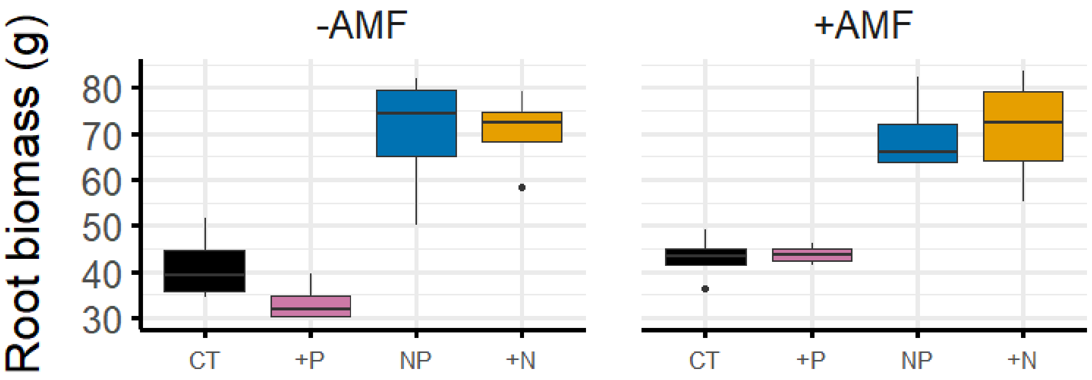
Figure 2.7 a) *Stem height growth and* ***b)*** *stem diameter growth of poplar and willow trees inoculated with arbuscular mycorrhizal fungi (+AMF) or without fungal inoculation (-AMF) in soils amended with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+NP) compared to unfertilised soils (CT); relative growth rates over one growing season are shown, boxes denote the 25th and 75th percentiles and median lines are given for n = 12, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5× the interquartile range, and dots indicate outliers.*

Destructive measurements of two pots per species revealed that N-fertilization increased leaf mass 240% ($F_{2,29}$ = 53.7, p < 0.001) and root biomass by 71% ($F_{2,29}$ = 49.8, p < 0.001) but there was no effect of fertilization with P or inoculation with AMF. Woody biomass increased by 56% with N-addition ($F_{4,27}$ = 9.6, p < 0.001), but the increase relative to the controls was only 26% in trees inoculated with AMF (N × AMF interaction: p = 0.078; Figure 2.8c). Total biomass varied with both N-fertilisation and AMF-inoculation ($F_{4,27}$ = 9.6, p < 0.001), whereby biomass was significantly higher in N-fertilised trees (p < 0.001) and in AMF-inoculated trees without N-addition (p = 0.032), but inoculation with AMF reduced the effect of N-fertilisation (N × AMF interaction: p = 0.072; Figure 2.8d) (Appendix A1).

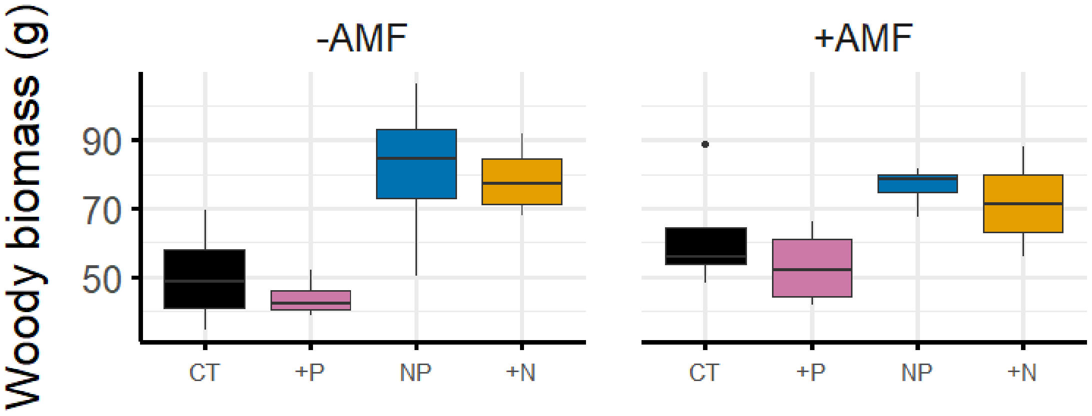
a)


-AMF
+AMF
Leaf mass (g)
40
30
20
10
CT
+P
NP
+N
CT
+P
NP
+N

b)


-AMF
+AMF
Root biomass (g)
80
70
60
50
40
30
CT
+P
NP
+N
CT
+P
NP
+N

c)


-AMF
+AMF
Woody biomass (g)
90
70
50
CT
+P
NP
+N
CT
+P
NP
+N

d)

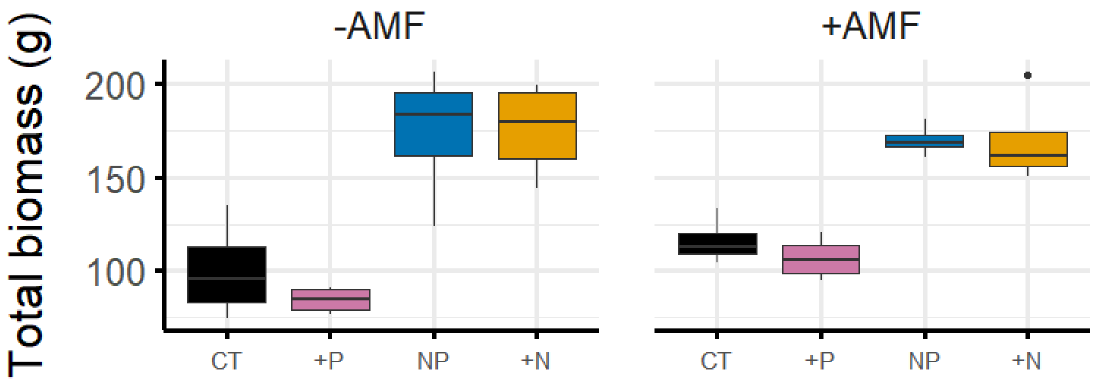


Figure 2.8 *Biomass of **a)** leaves, **b)** roots, **c)** stems and branches and **d)** total biomass of trees (willow and poplar) inoculated with arbuscular mycorrhizal fungi (AMF+) or without fungal inoculation (AMF-) in soils amended with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+NP) compared to unfertilised soils (CT) after one growing season; boxes denote the 25th and 75th percentiles and median lines are given for n = 2, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

Nutrient analysis

At the end of year one there was no treatment effect on ammonium-N, nitrate-N, total inorganic N or total N in soils under either poplar or willow. P addition significantly reduced P levels in soils under willow (F= 5.52, p=0.02; Figure 2.9) but had no effect on soils under poplar.

There was no effect of any single treatment on TOC in soils under willow. However the interaction between AMF addition and N addition significantly decreased TOC in soils under poplar (F=4.98, p=0.029; Figure 2.10). There was no effect of any single treatment on the CN ratio of soils under either poplar or willow (Appendix A2).

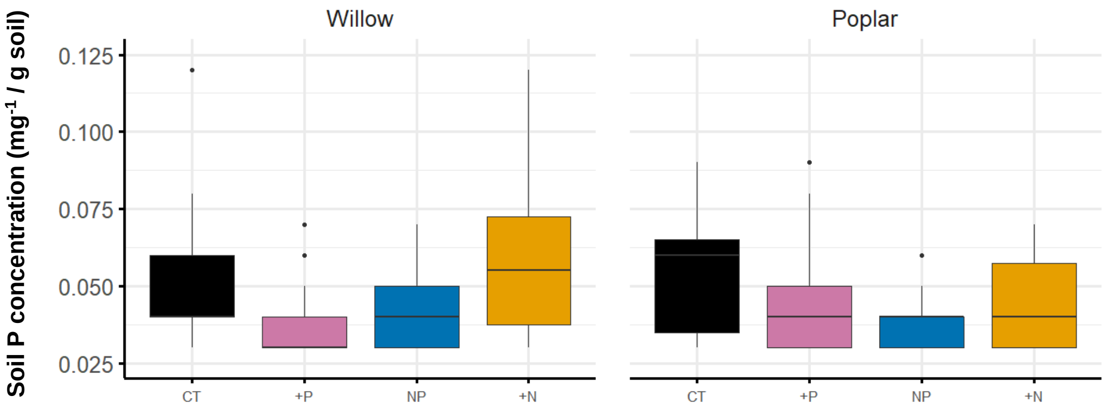


Figure 2.9 *P concentration of soils under willow and poplar inoculated with arbuscular mycorrhizal fungi (AMF+) or without fungal inoculation (AMF-) in soils amended with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+NP) compared to unfertilised soils (CT) after one growing season; boxes denote the 25th and 75th percentiles and median lines are given for n = 24, where n is the number of replicates per treatment*

combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.

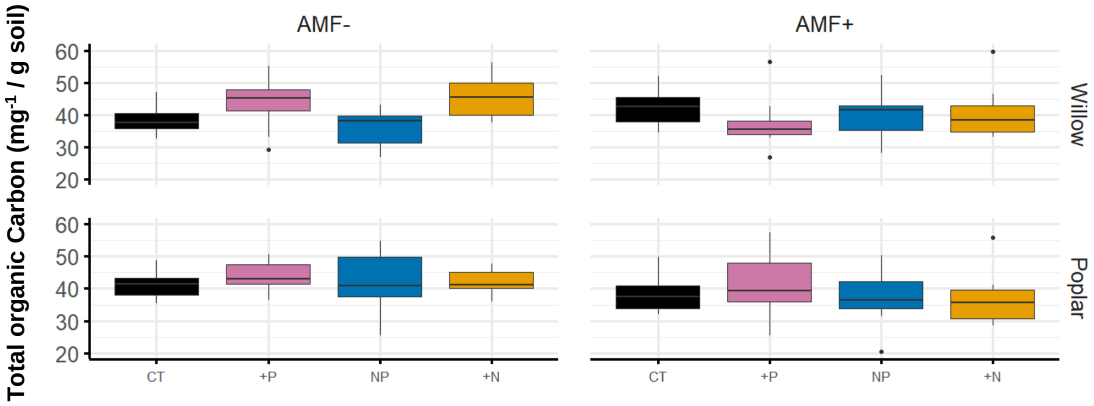


Figure 2.10 *Total organic carbon of soils under willow and poplar) inoculated with arbuscular mycorrhizal fungi (AMF+) or without fungal inoculation (AMF-) in soils amended with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+NP) compared to unfertilised soils (CT) after one growing season; boxes denote the 25th and 75th percentiles and median lines are given for n = 12, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

Soil respiration

Soil respiration rates were unaffected by P addition but increased with N addition under both tree species (Figure 2.11; Appendix A3). The effect of N addition on soil respiration rates was particularly prominent during the second growing season, when respiration in N-fertilized pots (poplar: 6.5 ±0.3 μmol CO_2 m^{-2} s^{-1}; willow; 4.95 ±0.1 μmol CO_2 m^{-2} s^{-1}) was almost twice as high as in pots without added N (poplar: 2.75 ±0.1 μmol CO_2 m^{-2} s^{-1}; willow: 2.695 ±0.4SE μmol CO_2 m^{-2} s^{-1}). Soil respiration under willow was 88% higher with N-fertilisation ($\chi^2 = 35.4$, $p < 0.001$) but AMF inoculation had no effect. Soil respiration under poplar also increased 1.4-fold with N-fertilisation and although AMF inoculation had no effect on respiration under N-fertilised trees, respiration under unfertilized trees increased by 29% with AMF inoculation (N × AMF interaction: $\chi^2 = 36.5$, $p < 0.001$). Hence, the increase in soil respiration with N-addition relative to controls was greatest under uninoculated trees (Figure 2.12; Appendix A3).

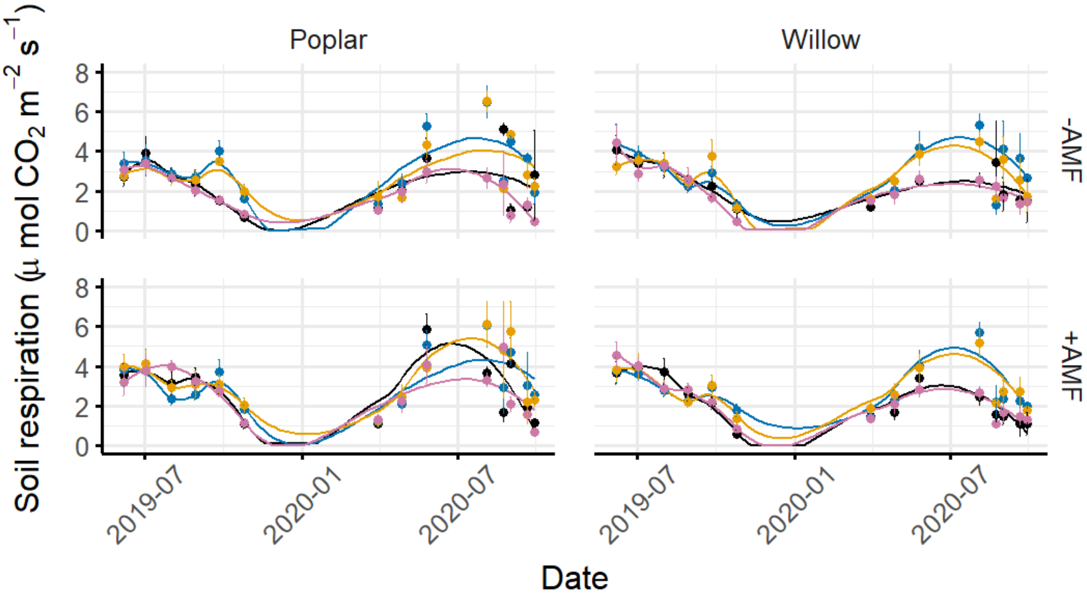


Figure 2.11 *Soil respiration under poplar and willow trees inoculated with arbuscular mycorrhizal fungi (AMF+) or without fungal inoculation (AMF-) in soils amended with nitrogen (yellow), phosphorus (pink), nitrogen and phosphorus (blue) compared to unfertilised soils (black); respiration rates are shown over two growing seasons; dots and whiskers represent means and standard errors for n = 12, where n is the number of replicates per treatment combination per tree species,.*

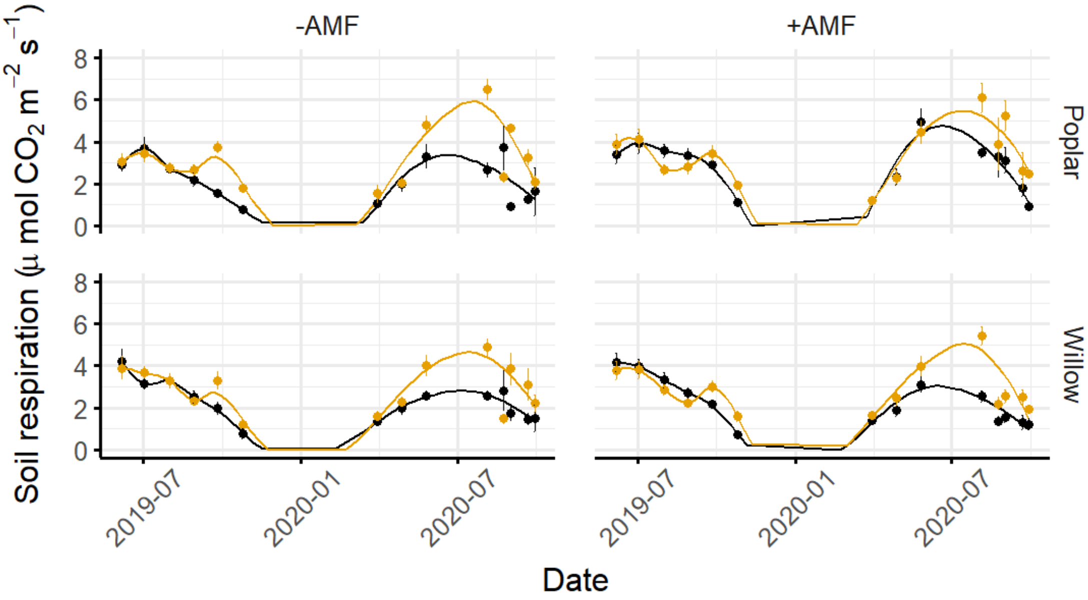


Figure 2.12 *Soil respiration under poplar and willow trees inoculated with arbuscular mycorrhizal fungi (AMF+) or without fungal inoculation (AMF-) in soils amended with nitrogen (yellow), compared to unfertilised soils (black); respiration rates are shown over two growing seasons; dots and whiskers represent means and standard errors for n = 12, where n is the number of replicates per treatment combination per tree species,.*

2.4 Discussion

My study demonstrated that interactions between fertilization and AMF inoculation of SRC crop species can influence plant growth, biomass, and soil C dynamics. Importantly, inoculation with AMF also enhanced plant growth but reduced some of the biomass gains from N-fertilisation. Although the effects of N and P addition to soils are likely to be soil-and site-specific, the findings from my common-garden experiment provide a solid foundation for future field studies. Here, I interpret my findings within the context of bioenergy crop management and discuss their broader implications.

AMF colonisation

The lack of evidence for AMF colonisation, despite clear effects of AMF inoculation on growth and respiration, are probably due to the extent or timing of root sampling. The extent of root sampling can influence estimates of colonisation because root colonisation by AMF is a continuous process, and arbuscules have relativity high turnover rates. Consequently, at any one time, some roots will show evidence of colonisation while other roots will not. However, overall colonisation across the root system would remain fairly constant (Smith and Read, 2008d) and it is unlikely that only uncolonized roots were sampled by chance. The timing of root sampling is a more likely explanation because I sampled at the end of the growing season. Mycorrhizal fungi are drawn to young roots by hormone release and roots lignification with ageing protects the cortex from penetration (Brundrett, 2002). In addition, AM fungi show seasonal changes in colonisation, with autumn and winter having lowest levels. As I performed my sampling at the end of the growing season, colonisation levels would have been low (Escudero and Mendoza, 2005). However, it is important to note that other plant processes involving AMF have been measured without evidence of current colonisation (Küpper et al., 2001). High initial concentrations of P in the soil might also explain the lack of evidence for AMF colonisation. AM fungi mainly improve P availability to plants, and there is thus little incentive for plants to provide the fungal partner with C when they are not limited by P availability (Fitter, 1991).

Tree growth and biomass

In partial support of my first and second hypotheses, stem diameter growth and woody biomass increased with AMF inoculation and N addition but not with P addition. The substantial increase in the growth and biomass of both tree species with N addition demonstrates the high N requirement of fast-growing, high-yield SRC crop species such as willow and poplar (Djomo, Kasmioui and Ceulemans, 2011). Although low fertiliser requirement is one of the perceived benefits of both willow and poplar, N-addition can substantially increase yield (Aronsson, Rosenqvist and Dimitriou, 2014a). While UK government current recommendations state that no fertiliser addition is required during establishment of the crop, my study shows that N-addition during crop establishment might be beneficial for achieving high yields rapidly. The particularly large gains in root biomass with added N (Figure 2.8b) reflects the young age of the trees in my study, because the effects of N fertilisation on root biomass are generally more pronounced in young plantations where investment of resources in rapid root growth enhances plant survival (Janssens et al., 2010). As bioenergy crops are fast-growing, C sequestration in woody biomass is also often high (Rytter, Rytter and Högbom, 2015). Under sufficient N supply, the plants allocate more resources to aboveground biomass and create larger canopies, which also increases their C sequestration capacity (Janssens et al., 2010). Overall, my findings demonstrate substantial growth and biomass increases with N addition in both species, and thus, that N fertilisation could enhance C sequestration in bioenergy crops. However, this gain in C could be offset by the C footprint of fertiliser application, including enhanced release of N_2O after N fertilization. A full life-cycle assessment is needed to reveal whether additional C sequestration in N-fertilised SRC crops offsets the embedded C footprint of fertiliser application.

Importantly, AMF inoculation increased stem diameter growth in both tree species, and woody biomass in unfertilized trees (Figures 2.7b and 2.8c). However, in contrast to my second hypothesis, the interactive effects of N-fertilisation and AMF inoculation varied between species. Although the combined effects of N-fertilisation and AMF inoculation boosted growth in poplar trees, AMF inoculation tended to reduce the stimulatory effects of N-fertilisation in willow. The smaller biomass gains in N-fertilised trees inoculated with AMF are likely due to reduced height growth of inoculated willow trees with added N (Figure 2.7a). High N requirements of the fungal partner (Hodge and Fitter, 2010) could explain reduced height growth of AMF-inoculated willow trees with added N via two possible mechanisms: 1) the fungal partner outcompeted the trees for some of the added N; and 2) N addition stimulated AMF growth, placing greater demands for C on the plant partner. Either of these mechanisms would reduce plant C investment in biomass growth. However, it is noteworthy that AMF-inoculation enhanced stem diameter growth in both species, and woody biomass in unfertilized trees, indicating that the fungal partner improved plant nutrient acquisition to the extent that aboveground C allocation increased. These findings suggests that AMF inoculation could benefit SRC bioenergy crops on nutrient-poor soils or reduce overall fertilizer need.

Addition of P had no influence on tree growth or biomass in my study, even though recent research on poplar and willow demonstrated increased leaf biomass with P addition (Da Ros et al., 2018a). It is possible that the levels of P in the soil met tree requirements during the first year of growth, and both tree species can store excess of P in their leaves (Da Ros et al., 2018a). However, it is noteworthy that the levels of available P in fertilised soils at the end of the growing season were no different to the controls. Although luxury consumption of P by the trees could account for high P uptake without concomitant gains in biomass (Bennett and Adams, 2001), it is also possible that surplus P fertilizer was leached from the soils. Although both species are often used to reduce nutrient leaching into watercourses by planting them in areas with fertiliser runoff, P leaching from natural poplar and willow stands into the surrounding environment can still occur (Da Ros et al., 2018a).

As AMF are particularly efficient at acquiring P for the host plant (Hodge, Helgason and Fitter, 2010), high background availability of P in the soil could explain why AMF inoculation had only limited effects on tree biomass. Nonetheless, P is relatively immobile in the soil compared to N, and P depletion zones around roots are common (Schachtman, Reid and Ayling, 1998). Given the distinct mobilities of N and P, plants grown in pot studies frequently suffer from P limitation at the start of growth, when the root system is still underdeveloped (Wissuwa, Gamat and Ismail, 2005; Hermans et al., 2006; Yang et al., 2010). However, rapid root growth in both species would have allowed the trees to overcome any such initial limitation. Continued growth of the trees in pots is likely to result in P limitation once existing soil reserves are depleted, and mycorrhizal hyphal networks would provide no additional benefit to potted plants because they are unable to extend further into the soil to acquire additional P. It is therefore possible that P limitation of growth or biomass in unfertilised trees will become apparent at the end of the second growing season.

Soil respiration and soil carbon

In partial support of my third hypothesis, soil respiration increased in AMF-inoculated poplar. However, soil respiration increased with N addition, but I did not observe the expected increase in respiration with P addition. Soil respiration is closely related to changes in root biomass and therefore, increased soil respiration rates with added N likely reflects the substantial increase in root biomass in N-fertilised trees (Figure 2.11, Figure 2.12). Increased root-rhizosphere respiration would also be expected due to the rapid growth of N-fertilised trees (Moscatelli et al., 2008; Rewald, Kunze and Godbold, 2016). The trees in my study were less than three years old, and the increased soil respiration can be attributed to a highly active root network and rapid establishment of aboveground biomass and leaf area (Jaoudé, Lagomarsino and de Angelis, 2011; Lagomarsino et al., 2013; Berhongaray et al., 2017; Ventura et al., 2019). Although added N can also stimulate the turnover of soil organic C and boost heterotrophic respiration rates (Zhou et al., 2014; Rewald, Kunze and Godbold, 2016), I observed no changes in soil TOC content with N-fertilisation (Figure 2.10), suggesting that the increase in soil respiration was largely autotrophic, or that losses of soil C were compensated by increased inputs from roots and rhizosphere microbial communities. Hence, although N addition often increases soil respiration (Janssens et al., 2010), the benefits of N addition for SRC crop growth indicate that the higher soil respiration rates will be offset by increased C allocation to biomass (Jaoudé, Lagomarsino and de Angelis, 2011). My findings therefore highlight the importance of understanding above- and belowground C dynamics in SRC plantations.

There was a clear effect of AMF inoculation on soil respiration under unfertilised poplar trees (Figure 2.11), but not under willow trees, indicating that species differences in plant-soil interactions can influence C cycling in bioenergy crops. Any changes in respiration due to AMF inoculation in fertilised trees were probably obscured by the substantial increase in respiration with N addition (Figure 2.12). As fertiliser addition reduces the need for the plant to invest in mycorrhizal associations (Ven et al., 2019), higher respiration rates with AMF-inoculation in unfertilised trees could indicate greater investment of C by the plant in the fungal partner to acquire nutrients. Increased allocation of C to AMF would boost soil respiration in two ways: first, mycorrhizal networks have a larger surface to volume ratio than roots and can contribute substantially to total soil respiration (Ven et al., 2019; Ventura et al., 2019; Lang et al., 2020). Second, rapid turnover of fungal mycelium provides a C source to decomposer organisms in the soil, boosting microbial respiration (Talbot, Allison and Treseder, 2008; Lin et al., 2017; Keller and Phillips, 2019; Fang et al., 2020b). Nonetheless, the decline in soil organic C in pots inoculated with AMF in my study indicates that increased respiration rates could also be due to fungal priming of SOM (Staddon et al., 2003; Driver, Holben and Rillig, 2005; Paterson et al., 2016; Lang et al., 2020), whereby new C inputs stimulate the mineralisation and release of older soil organic C (Frey, 2019).

It is noteworthy that TOC content was lower in soils under poplar that were both inoculated with AMF and fertilized with N (Figure 2.10). Several studies have demonstrated the capacity of AMF to induce a SOM decomposition priming effect, which could be enhanced if fungal N limitation is alleviated by fertilisation (Treseder and Allen, 2002). AMF hyphal networks are extensive, and it is thought they can account for 20-30% of total soil biomass (Donnelly, Boddy and Leake, 2004), thus creating large pools of labile C within the soil. Coupled with this, AMF communities have a high turnover rate of mycelium, which provides saprotrophic fungi with additional easily decomposable organic matter, providing a source of organic material for C release by decomposition, thus creating the priming effect (Fang et al., 2020a). Increased root exudation as a signal between roots and AMF also releases additional labile C into soil, which can boost SOM decomposition through changes in the soil microbial community (Shahzad et al., 2015). However, it is important to note that AMF offer numerous benefits to host plants such as overall improved plant health and ability to mitigate changing abiotic conditions (Smith and Read, 2008c), and potential C losses through priming by AMF are likely to be offset by enhanced plant health and growth.

It is notable that AMF inoculation had a much greater effect on growth, woody biomass and soil respiration in poplar trees compared to willow. Although AMF are generalist species, able to colonise up to 90% of vascular plants (Smith and Read, 2008b) including poplar and willow, it is possible the AMF community used was more suited to poplar than willow, contributing to the greater effect observed in poplar in my study. However, poplar and willow reach maturity at different ages and undergo senescence at different points in autumn. Leaf litter abundance varies and decomposition occurs at varying rates (Marler, Stromberg and Patten, 2001) and thus, differences in soil communities and respiration could be expected over the course of the lifecycle. In addition, willow and poplar have different growth dynamics: poplar can produce larger stem diameter, height, and wood density, while willow develop a greater number of shoots from sprouting, especially during early growth (Huber, Matiu and Hülsbergen, 2018). Hence, the effects of AMF inoculation on yield may be more apparent in poplar. Finally, poplar have a higher temperature requirement than willow (Kasanen, 2021) and AMF can enhance plant biomass with increasing soil temperature (Heinemeyer and Fitter, 2004). Hence, higher temperatures in the polytunnels may have favoured poplar trees inoculated with AMF.

2.5 Conclusions

Numerous studies have shown that land-use history, soil type and climate conditions can alter C cycling of bioenergy crops but none to my knowledge have assessed the potential benefits of mycorrhizal inoculation. I showed that although N addition in both tree species and AMF inoculation in unfertilised poplar trees increased soil respiration, the additional C release from the soil will be dwarfed by the considerable gains in plant biomass C where N addition is implemented. Although the effects of AMF inoculation and fertilisation in the field will be strongly dependent on site and soil conditions, my common-garden study nonetheless highlights the need for further in-depth assessment of how fertiliser addition and mycorrhizal colonisation of bioenergy crops might impact upon the C balance of SRC bioenergy crops. Harnessing mycorrhizae to establish SRC crops on marginal land requires further work to provide mechanistic explanations for the marked differences in the effects of AMF inoculation between species and the decline in soil organic C content under AMF-inoculated poplar that were fertilised with N. Field studies which incorporate differences in plant species and interactions between mycorrhizal species and nutrients would offer insights into balancing fertiliser use for increasing yield while minimising greenhouse emissions and nutrient leaching.

3. Carbon dynamics of willow & poplar amended with nutrients and mycorrhizal inoculation after an extreme drought event

3.1 Introduction

Globally, anthropogenic climate change has led to an increase in extreme weather, including more severe or more frequent drought (IPCC, 2021). Drought can be described as the result of inequality of the water fluidity rate between evapotranspiration (demand) and water transport into the soil-root interface (Lipiec et al., 2013). The impact of drought varies regionally but temperate climates are experiencing an increase in intensity and frequency of drought-rewetting cycles (DRW), where summers are warmer and drier, while winters are warmer and wetter (IPCC, 2021), which is affecting agricultural productivity and ecosystem carbon storage (Lorencová et al., 2013). While the drying and rewetting of soil is a normal seasonal occurrence, the changing frequency and intensity of these cycles are of increasing concern because they not only affect plant growth but also alter soil processes (Davidson, Belk and Boone, 1998) leading to land degradation, and altered soil carbon dynamics (Fierer and Schimel, 2002; Li et al., 2018; Rodríguez et al., 2019; Zhang et al., 2020). Importantly, increasing drought frequency reduces ecosystem carbon storage by limiting plant growth and thus plant carbon inputs to soils. As drought progresses, photosynthesis, root exudation and soil respiration decline (Ruehr et al., 2009). In addition, the drying of soil during a drought event followed by rewetting (DRW) by rainfall leads to a pulse in CO_2 emissions through the decomposition of dead matter and causes the mineralisation of soil carbon by microbial activity. First described by H.F Birch in 1958, the pulse of CO_2 released from soils is referred to as the "Birch effect" (Birch, 1958). A rewetting period after drought can produce a five-fold increase in CO_2 efflux compared to pre-drought values (Ficken and Warren, 2019). Such respiration pulses after a DRW event appear to peak within a few days and respiration rates return to pre-drought levels within two weeks (Bapiri, Bååth and Rousk, 2010). However, although numerous studies highlight the impact of DRW events on soil functions and soil respiration (Fierer and Schimel, 2002; Muhr et al., 2008; Rodríguez et al., 2019; Zhang et al., 2020; Hu et al., 2021), the impacts of drought on soil carbon dynamics after the event are still debated. For example, some studies found that drought reduces respiration to such an extent that even peak CO_2 release during Birch effects can be below pre-drought respiration rates (Ficken and Warren, 2019), whereas other studies provide clear evidence that pre-drought respiration rates are attained after only a few days (Li et al., 2018). The timing and magnitude of CO_2 release by Birch effects is likely to depend on the severity of the drought and the availability of carbon and nutrients in the soil (Jarvis et al., 2007; Lopez-Sangil et al., 2018).

Drought-rewetting cycles can also affect carbon storage potential in plants (Jarvis et al., 2007) through the release of stored soil carbon and the increased availability of nutrients and water upon rewetting. Indeed, the effects of DRW cycles on plant nutrient availability have received considerable attention because of the potential importance for agricultural crops (Bloor and Bardgett, 2012; Lorencová et al., 2013; He and Dijkstra, 2014). However, the inverse - the influence of nutrient availability on soil carbon release during DRW events - is still poorly characterised. Drought reduces plant availability of both nitrogen and phosphorus (P), but rewetting can increase nutrient availability above pre-drought conditions, indicating that sudden changes in soil moisture content greatly influence nutrient availability to plants (He and Dijkswtra, 2014). The length of the drought period and the intensity of the subsequent rewetting also have an impact on nutrient pulses in the soil (Jarvis et al., 2007). However, in conjunction with reduced plant uptake during drought, intense rewetting events could also flush mobile inorganic sources of nutrients from the soil, further impacting the recovery of the system from drought (He and Dijkstra, 2014). Interestingly, the reduction in plant nitrogen (N) and phosphorus (P) uptake and the N:P ratio of plant tissue is greater than the decline in plant growth during prolonged drought (He and Dijkstra, 2014), highlighting the importance of understanding how fertilization interacts with DRW events. Research into the role of nutrient availability in controlling CO_2 efflux during DRW events is especially important within agricultural ecosystems, as many crops are heavily reliant on fertilisation (Seufert and Ramankutty, 2017; Lacher et al., 2019).

Although the impact of DRW cycles on nutrient availability in global cash crops, such as maize, soybean, potatoes, barley, rice and wheat, has been extensively studied (Wraith, Baker and Blake, 1995; Ahmadi et al., 2010; Canarini and Dijkstra, 2015; Cerezini et al., 2019; Zhu et al., 2019; Bi et al., 2020), we know very little about how changes in precipitation will affect important bioenergy crops such as short rotation coppice (SRC) plantations. SRC crops are documented as having a high water requirement, thus making them ideal for use in areas of excessive soil water (Dimitriou, Busch and Jacobs, 2009; Oliver, Finch and Taylor, 2009; Aylott et al., 2010; Barnes et al., 2018; Mickan et al., 2019). This high water requirement makes them vulnerable to drought, and the effects of water limitation are likely to be exacerbated with prolonged drought periods, where AMF colonisation is reduced (Compant, Van Der Heijden and Sessitsch, 2010b). Understanding the impacts of DRW events in SRC crops is crucial, because the establishment of SRC crops has been widely encouraged on marginal agricultural land, which is often characterised by degraded soils and flooding risk (Headlee, Soolanayakanahally and Richardson, 2020), and the carbon sequestration capacity of SRC crops is a major incentive for their establishment (Rowe, Street and Taylor, 2009; Amichev et al., 2012; Lockwell, Guidi and Labrecque, 2012). The carbon-neutral status of SRC is achieved through rapid carbon allocation to woody biomass (Verlinden et al., 2013), which is affected by changes in water availability. Although SRC species such as willow or poplar are resistant to short-term drought, an intense drought period during the growing season is likely to impact woody biomass and canopy growth of deciduous tree species (Lobo-Do-Vale et al., 2019). Both willow and poplar can develop premature maturing of woody biomass under drought, identified by the early colouring of young stems and premature leaf drop (Larchevêque et al., 2011; Köhler et al., 2020). The differences in drought tolerance between species is less commonly assessed than the differences between genotypes within species. However, studies show that although willow generally has a greater yield, it also has a lower drought tolerance than poplar (Cochard, Casella and Mencuccini, 2007). Similarly, differences in drought tolerance within species are often related to genotype, with high-yield varieties having a lower drought tolerance than lower yield varieties (Wikberg and Ögren, 2007; Larchevêque et al., 2011; Khan et al., 2016; Jia et al., 2020). However, as nutrient availability and species interactions affect tree growth rates, they also have the potential to influence drought tolerance of both species.

The mutualistic relationship between SRC crop species and arbuscular mycorrhizal fungi (AMF) could influence the resistance and resilience of the entire system to DRW events. A substantial number of studies has established that colonisation of plants with AM fungi can mitigate drought periods (Augé, 2001), where colonised roots are able to obtain and uptake moisture both outside of the root zone and in previously inaccessible pores (Diagne et al., 2020), while also improving plant N and P uptake and water retention (Li et al., 2014; Liu et al., 2018). Local AMF communities can also increase potassium (K) exchange rates in soils during a drought event (Marulanda et al., 2007). More recently, studies focussing on the importance of AM fungal colonisation of plants in mitigating DRW events have highlighted the importance of improved nutrient uptake and water accessibility (Liang et al., 2018). The prevalence of mycorrhizal fungi can also influence the Birch effect (Jarvis et al., 2007) because arbuscular mycorrhizal fungi (AMF) have high mycelial turnover (Staddon et al., 2003). Mycelial death and decay during drought can maintain soil respiration rates, followed by a delay in the recovery of soil respiration as the fungal community regenerates, which would explain a lag in Birch effects upon rewetting in AMF-dominated soils (Ficken and Warren, 2019). While AMF respiration is sensitive to drought events, the production of the fungal protein glomalin appears to reduce this sensitivity in comparison with other soil microbes (Ficken and Warren, 2019) and AMF growth does not appear to be impacted (Bapiri, Bååth and Rousk, 2010). In addition, although drought alters the structure and composition of the soil microbial community, it does not appear to reduce the transfer of carbon from plant roots to AMF (Bapiri, Bååth and Rousk, 2010). In fact, soils under drought conditions can show increased fungal population density (Fuchslueger et al., 2014). However, mycorrhizal fungi are likely to experience abiotic stresses independently of their host plant (Millar and Bennett, 2016), and thus the impacts of DRW events on soil carbon dynamics are regulated by the independent responses of plants and the soil microbial community as well as the interactions between them (Ficken and Warren, 2019). Importantly, the impact of DRW events will depend on the predominant AMF community formed during colonisation of plants, as this not only determines the fungal response to DRW events but also influences how the plants respond (Braunberger, Abbott and Robson, 1996). However, the extent to which plants or mycorrhizal fungi contribute to Birch effects upon rewetting after drought is not known (Ficken and Warren, 2019). In addition, the movement and allocation of carbon during a DRW event is plant-species dependent (Ficken and Warren, 2019) and hence the relationship between different plant species and their mycorrhizal partners is likely to influence the impact of drought on carbon and nutrient dynamics in SRC crops.

The impact of DRW events on carbon storage by SRC crops is likely to be influenced by the crop species, their mycorrhizal partners, and the availability of nutrients. However, a lack of understanding about the interactions between nutrient availability, fungal communities, and plant reactions to drought conditions make estimating carbon cycling under future climate scenarios extremely challenging (Ficken and Warren, 2019). It is vital we address this to understand how climate change induced abiotic stresses alter soil processes and how we must adapt soil management practices to mitigate this (Hobbs and Govaerts, 2010). Hence, knowledge of how each of these components and their interactions influence carbon dynamics during recovery from drought can inform the establishment and management of SRC plantations on marginal land. In this study, I aimed to assess how fertilization and mycorrhizal inoculation influence soil respiration and nutrient availability under established SRC crop species willow (*Salix purpurea*) and poplar (*Populus nigra*) after a strong drought event. I grew willow and poplar saplings in mesocosms with added N, P, and a commercial mycorrhizal inoculum for a full year before subjecting them to a drought and a rewetting event. I measured plant growth rates, biomass, soil CO_2 efflux and soil nutrient availability to test the following hypotheses:

H1) Rewetting after drought will cause a strong transient increase in soil respiration (Birch effect), but fertilization with N will reduce the magnitude of Birch effects.

H2) Inoculation with AMF will delay the onset of Birch effects.

H3) Rewetting after drought will improve the availability of nutrients but the magnitude of the rewetting effect will differ between tree species and AMF inoculation treatments.

3.2 Methods and materials

Experimental design

To establish how a drought event interacts with AMF inoculation and nitrogen treatments to influence SRC bioenergy crop yield and plant–soil carbon dynamics, I used a sub-sample of an established large pot study (Chapter 2) with *Populus nigra* L. (poplar) and *Salix purpurea* L. (willow) trees, which are both commonly planted as bioenergy crops on marginal land. In May 2019, I set up 192 18-L pots (30-cm diameter, 30-cm height) in two polytunnels at the James Hutton Institute, Dundee, Scotland. Each polytunnel was split into three blocks per species (six replicate blocks per species in total), and 16 pots were assigned to each block. Each pot was filled with 17.5-L of pre-sterilised sandy loam soil, made from a 1:1 mix of commercial loam (Keith Singletons, Cumbria, UK) and sand, which had been autoclaved for 2 h. Half of the pots per block were inoculated with a commercial AM fungal inoculant (AMF+), whereby each pot was layered with 5-L sterilised soil, followed by 7.5 L soil mixed with 50-g rootgrow^{TM} granules (PlantWorks Ltd, Kent, UK), and then another 5 L of sterilised soil. The other half of the pots were left as uninoculated controls (AMF-).

I grew poplar (The Poplar Tree Company, Madley, UK) and willow (The Willowbank, Lynbrook, UK) from 30-cm cuttings. I planted 100 cuttings of each species in Deepots^{TM} cells (6-cm diameter, 25-cm depth; Stuewe & Sons Inc, Oregon, USA) with 650 ml autoclaved sandy loam. After four weeks of growth, 96 randomly selected cuttings were transferred to 18-L pots (16 pots per species per replicated block). Cuttings were removed from the Deepots^{TM}, the root ball gently rinsed with cold water, and then half the cuttings were planted in the +AMF pots, and half in the -AMF pots. The cuttings were planted to one side of the pot to allow space for soil measurements and all cuttings were pruned to ensure only the main stem was left. Each pot was watered automatically daily at 8.30 am and 3.30 pm for 90 seconds, using two pressure-compensated spray stakes (flow rate 0.2-L min^{-1}; Netafim Ltd., Skelmersdale, UK), using on-site mains water supply.

Spray stakes were located on opposite sides of each pot, with the spray directed towards the centre.

To measure soil CO_2 efflux, a permanent soil collar was installed in each pot. The collars were constructed of drainage pipe (11 cm diameter and 12 cm height) inserted c. 10 cm into the soil, leaving 2 cm above the soil surface (Floplast Ltd, Kent, UK). All collars were sterilised in 1% bleach solution for four hours, rinsed thoroughly and then installed slightly off centre on the opposite side to the tree in each pot, taking care to minimise soil disturbance and root damage.

Fertilization levels were chosen based on recommendations for bioenergy crop establishment, whereby 120 mg N L^{-1} yr^{-1} and 80 mg P L^{-1} yr^{-1} are considered a high level of fertiliser (Tubby and Armstrong, 2002; Sevel et al., 2014a,b; Georgiadis et al., 2017). To ensure the correct amounts of N and P were added to each treatment, I created a stock solution using different chemical compounds for each treatment. The +N pots each received 8.46 g KNO_3 and 2.21 g NH_4NO_3 per year. The +P pots each received 7.28 g K_2HPO_4, and the +NP pots received 5.67 g of NH_4NO_3 and 7.28 g of K_2HPO_4. Hence, each of the three fertilization treatments also added potassium (K) at a rate of 202 mg K L^{-1} yr^{-1}. The trees were fertilised in a single application in June 2020, to mimic management of bioenergy crops in the field.

Drought treatment

To assess how SRC crops recover from a DRW event and establish how this alters soil CO_2 emissions and nutrient availability, I imposed a drought treatment on four blocks per tree species, two from each polytunnel. After delays due to the Covid-19 pandemic lockdown, the study was undertaken between August 2020 and October 2020, allowing for measurements to be completed during the growing season while trees were fully active. At the start of the experiment, the trees were well established with no signs of distress, and baseline data included 18 months of tree growth and soil respiration measurements (Chapter 2). Half of the pots per treatment and block were assigned to a drought treatment and the remaining half continued to receive the pre-existing watering regime. Thus, the experiment included fertilization, AMF inoculation, and drought treatments in a factorial design, giving four replicates per species and treatment for a total of 16 treatments (two species × four fertilizer treatments × two AMF treatments × two drought levels = 128 pots in total).

In August 2020, watering of the pots assigned to the drought treatment was suspended for 28 days, which ensured that soil water holding capacity dropped to below 20% to create an effective drought (de Vries et al., 2012) but without killing the cuttings (Khan et al., 2016). Control pots continued to be watered twice daily via drippers as described above. After 28 days, the efficacy of the drought treatment was assessed by measuring soil water content horizontally through the middle of the sidewall of each pot, using a 10-cm soil moisture probe (Lutron PMS-714, Taipei, Taiwan). The soil water content in the drought pots was only 10% and all trees in the drought pots were showing clear signs of water deficit stress: willow trees showed premature autumn colour change and leaf drop, while poplar showed signs of drooping and leaf yellowing. All pots (drought and control treatments) were then rewet to field capacity, using 2L of water per pot, applied by top watering by hand. To assess recovery of soil water availability after drought, SWC was remeasured 1, 3, 7, 14, 21 and 28 days after rewetting.

Soil respiration

Soil respiration was measured in all pots immediately before the drought treatment using an automated soil gas flux system (Li-8100A; LiCOR Biosciences, Lincoln, Nebraska, USA) with a 10-cm diameter survey chamber. Each measurement lasted 2 mins with a 15-s pre-purge period and a 30-s dead-band (Chapter 2). Soil respiration was then re measured immediately after rewetting (day 0), and then after 1, 3, 7, 14, 21 and 28 days. The drought treatment, rewetting and respiration measurements were staggered by one week between polytunnels to allow sufficient time for high-frequency soil respiration measurements. On day 14 after rewetting, the watering regime was reinstated for all pots to prevent further drought. Tree growth and soil respiration measurements were made on all pots 28 days after rewetting, after which, soil respiration measurements continued monthly. Soil CO_2 efflux was calculated using the SoilFluxPRO software (LiCOR Biosciences, Lincoln, Nebraska, USA) Figure 2.4b).

Nutrient availability

To establish how drought and re-wetting altered plant-available nutrients, (N, P, K, Ca & Mg), I installed two anion and two cation exchange resin strips (PRS probes, Western AG, Canada) in each pot on day 14 after rewetting. After 14 days of exposure, the probes were carefully removed and gently cleaned of all remaining soil particles using dH_2O and a soft brush. All probes per pot were placed together in a plastic bag and shipped in temperature-controlled packaging to the manufacturer for analysis.

AMF colonisation

I determined AMF colonisation in the control pots when the trees were harvested (Chapter 4) after 25 months of growth: dried root samples were placed into tissue cassettes, cleared in 3% KOH at 102ºC for 25 minutes, acidified in 2% HCl for 30 minutes, and stained with 0.05% Trypan Blue for 30 minutes. The tissue cassettes were then stored in de-stain solution and refrigerated until analysis of root colonisation. Roots were removed from cassettes and placed on microscope slides with a minimum of ten roots per slide where possible. Slides were left to air dry for five days and then polyvinyl lactoglycerol (PVLG) was added to affix cover slips. I then assessed the proportion of root length colonised by AM fungal hyphae, arbuscules, vesicles and spores using a compound microscope and the gridline intersect method (McGonigle et al., 1990). Briefly 100 root fragments were assessed per slide by moving through the slide and assessing presence or absence of mycorrhizal structures in each fragment.

Data analyses

Data analyses were performed with R version 4.1.1 (R Core Team, 2021) in RStudio (RStudio Team, 2021), using the *lme4* (Bates et al., 2015), MuMIn (Barton, 2020), and lmerTest (Kuznetsova, Brockhoff and Christensen, 2017) packages. Results were graphed using the ggplot2 package (Wickham and Chang, 2016). Data were power- or log-transformed before analysis where necessary to meet modelling assumptions. For all analyses, the nutrient treatments were included as factorial N or P addition, whereby N-addition (+N and +NP treatments) was compared to no N-addition (CT and +P treatments) and P-addition (+P and +NP treatments) were compared to no P-addition (CT and +N). Given the differences in nutrient requirements and drought tolerance between poplar and willow, separate analyses were conducted for each species.

First, I assessed whether the four-week drought treatment had reduced soil respiration by comparing soil CO_2 efflux rates between droughted and un-droughted pots immediately before rewetting (day 0). I tested the effects of drought, factorial nutrient additions (with or without N or P), AMF inoculation, and their interactions on soil respiration before rewetting using linear models. As the models indicated that drought only reduced respiration in the pots with added N, factorial P additions were excluded from the analyses of post-drought respiration rates. I then tested whether the respiration peak upon rewetting differed between pots with N-addition, AMF inoculation and their interaction using separate linear models for each species. To assess whether AMF inoculation influenced soil respiration rates during the month after drought, I used linear mixed effects models with factorial N-addition, AMF inoculation and their interaction as fixed effects, and block and time (days since rewetting) as random effects. Finally, I determined the effects of the treatments on soil nutrient ion exchange rates using linear models. I modelled the exchange rates for each nutrient as a function of factorial nutrient additions (with or without N or P), AMF inoculation, drought, and their interactions.

The success of mycorrhizal colonisation was determined from counts of arbuscules and hyphae in roots harvested after two growing seasons using Wilcoxon rank sum tests to compare AMF+ and AMF- treatments for each species separately (Table B1).

All models were simplified by sequential removal of non-significant terms until the most parsimonious model remained. For linear mixed effects models, the models were simplified by sequential removal of terms, using AIC and p-values to assess model improvement (Pinheiro & Bates 2000; Appendix B). I used diagnostic plots to assess model residuals and the final models were compared to appropriate null models (intercept-only) using likelihood ratio tests. The significance of individual terms was determined using Satterthwaite's approximation to estimate degrees of freedom. I report the χ^2 and *p*-values from the likelihood ratio tests for the final model fit. All results are reported as significant at $p < 0.05$ and as trends at $p < 0.075$.

3.3 Results

Efficacy of treatments

On the final day of drought treatment, (day 0) SWC was significantly 31% lower in poplar ($t = -7.521$, $p < 0.001$) and 62% lower in willow ($t = -10.477$, $p < 0.001$) compared with control pots (Figure 3.1, Appendix B2). The addition of N further reduced SWC, resulting in 74% lower SWC in poplar ($t = -3.735$, $p < 0.001$) and 76% in willow ($t = -3.022$, $p = 0.003$) in droughted pots with added N compared to controls (Figure 3.1; Appendix B2). In poplar, the decline in SWC in drought pots without added N was significantly smaller than in drought pots with added N (drought × N interaction: $t = -4.790$, $p < 0.001$).

Upon re-wetting, a sharp increase in SWC was observed between day 1 and day 3, but SWC did not return to control levels even after 28 days (Figure 3.2; Appendix B3). Inoculation with AMF had no effect on soil water content (Appendix B3).

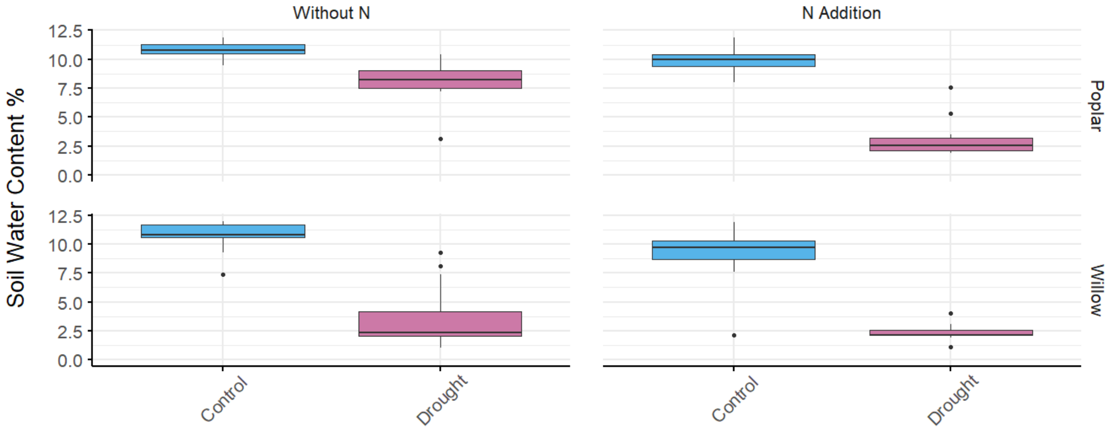


Figure 3.1 *Soil water content on the final day of drought (day 0 of rewetting) under poplar and willow trees in soils with nitrogen addition under a drought event (pink) or the standard watering regimen (blue) compared with unfertilised soils; boxes denote the 25th and 75th percentiles and median lines are given for n = 8, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

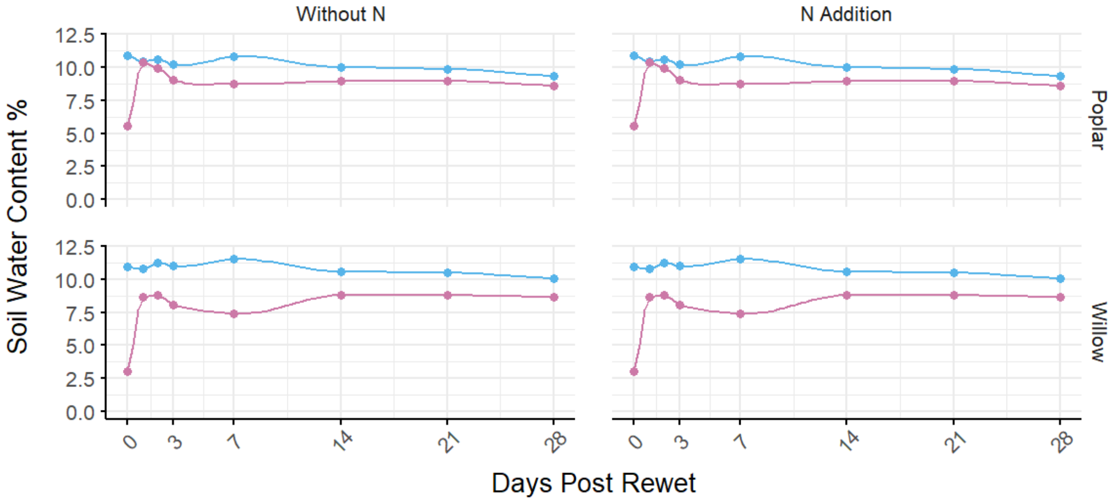


Figure 3.2 *Soil water content under poplar and willow trees following a drought event and rewetting (Drought) or established watering regimen (Control) in soils amended with nitrogen compared to unfertilised soils; volumetric soil water content is shown from start of rewetting until the end of the experiment 28 days later; dots represent means for n = 8, where n is the number of replicates per treatment combination per tree species, .*

The effects of the AMF inoculation treatment were clearly apparent after two growing seasons: greater numbers of arbuscules (Figure 3.3a) and hyphae (Figure 3.3b) were found in roots of AMF-inoculated poplar (*p* = 0.003 and *p* = 0.06, respectively) and willow trees (*p* < 0.001 and *p* = 0.002, respectively) compared to uninoculated trees, and a greater proportion of roots showed evidence of AMF presence (*p* < 0.001 for both species; Figure 3.3c, Appendix B1).

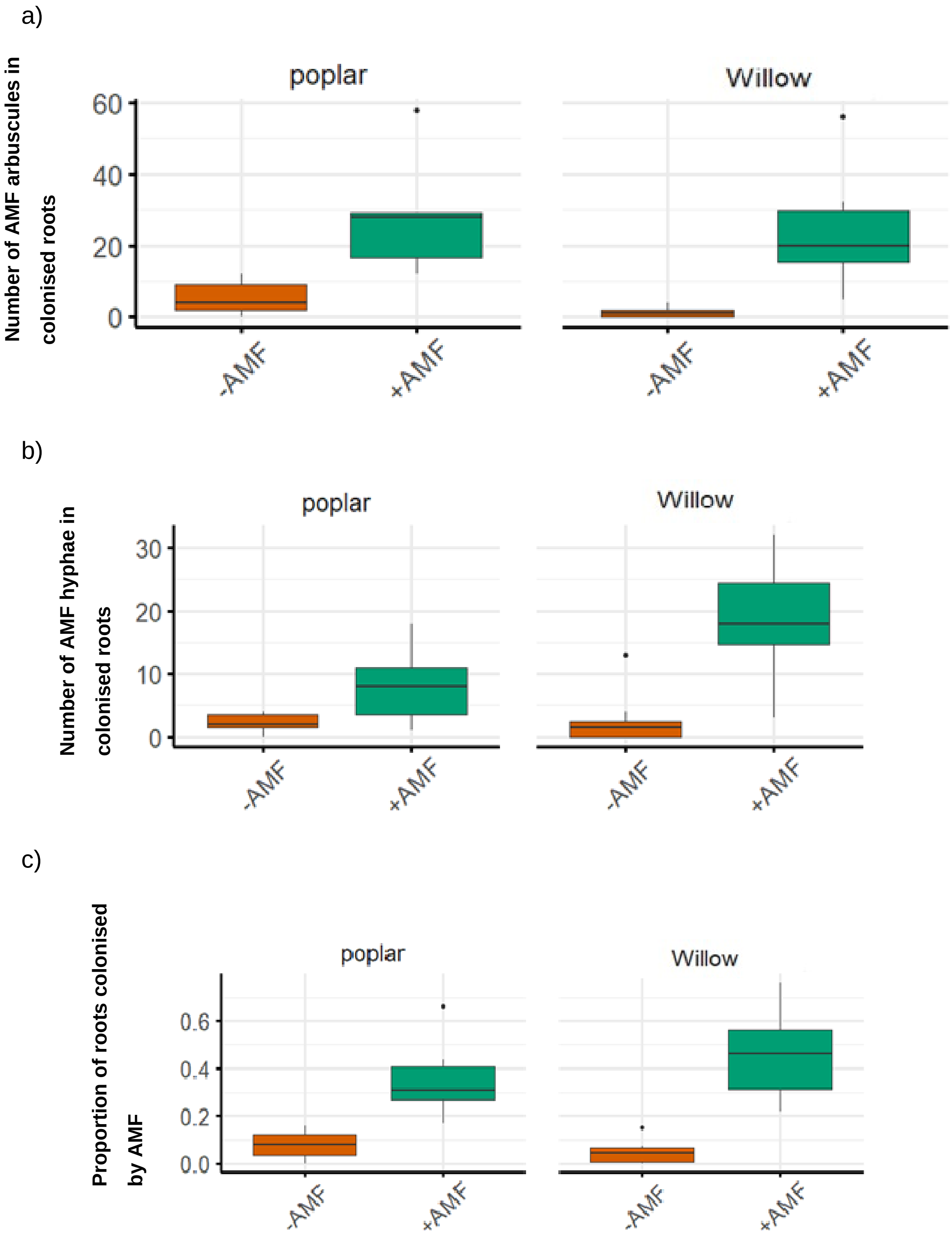


Figure 3.3 *The number of* ***a)*** *arbuscules and* ***b)*** *hyphae and* ***c)*** *the proportion of roots showing evidence of arbuscular mycorrhizal fungi (AMF) in poplar and willow trees inoculated with AMF (+AMF; green) compared to uninoculated controls (-AMF, orange) after two growing seasons. boxes denote the 25th and 75th percentiles and median lines are given for n = 32, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

Soil respiration

On the last day of the drought treatment, soil respiration under poplar was higher in pots with added N but neither drought nor AMF had an overall effect on soil respiration ($F_{3,124} = 6.50$, $p < 0.001$; Figure 3.4, Appendix B4). However, soil respiration under poplar was significantly lower in droughted pots with added N (N × drought interaction: $p < 0.001$). Soil respiration under willow showed a similar pattern, but the overall model was not significant (Figure 3.4, Appendix B4). Inoculation with AMF had no influence on soil respiration under drought in either species (Appendix B4).

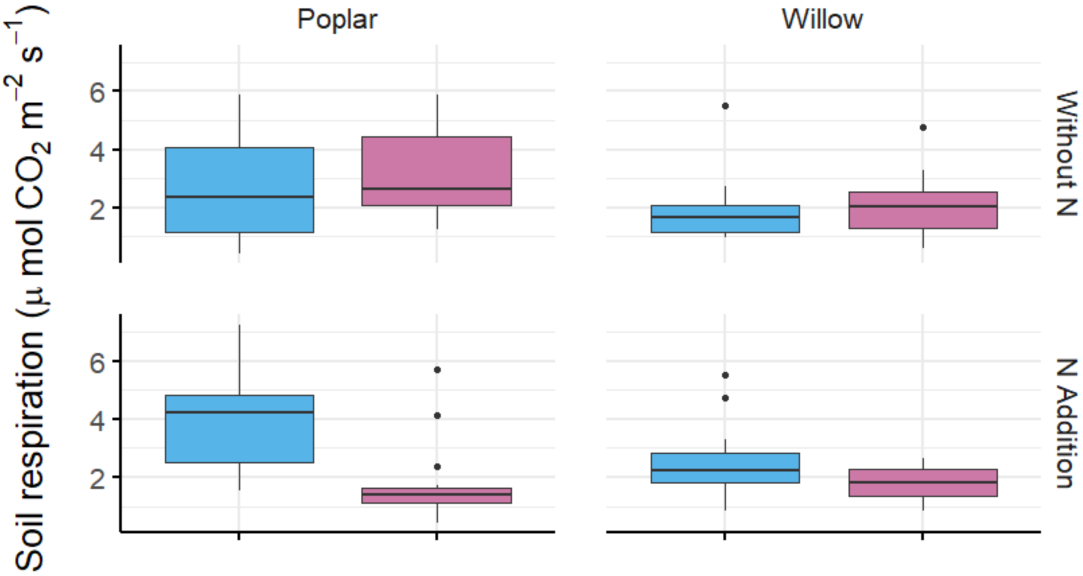


Figure 3.4 *Soil respiration on the last day of drought (day 0 of rewetting) under poplar and willow trees in soils with nitrogen addition under a drought event (pink) or the standard watering regimen (blue) compared with unfertilised soils; boxes denote the 25th and 75th percentiles and median lines are given for n = 16, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

The peak in soil respiration after rewetting was significantly higher with N-addition compared to pots without N under both species (poplar: $F_{1,62} = 21.1$, $p < 0.001$ and willow: $F_{1,62} = 14.4$, $p < 0.001$) but AMF inoculation had no effect (Figure 3.5a, 3.5b, Appendix B5). During the month after rewetting, the best model for soil respiration under poplar included the interaction between N-addition and AMF-inoculation ($\chi^2 = 21.8$, $p < 0.001$; Figure 3.5a). However, the individual effects of N-addition and AMF-inoculation were not significant, and the N × AMF interaction was weak ($p = 0.058$). Under willow, the pattern of soil respiration was also influenced by N-addition and AMF-inoculation ($\chi^2 = 57.7$, $p < 0.001$; Figure 3.5b), whereby soil respiration during the month after rewetting was higher with added N ($p < 0.001$) and AMF inoculation ($p < 0.001$), but N-addition reduced the stimulatory effect of AMF-inoculation (N × AMF interaction: $p = 0.005$; Appendix B5).

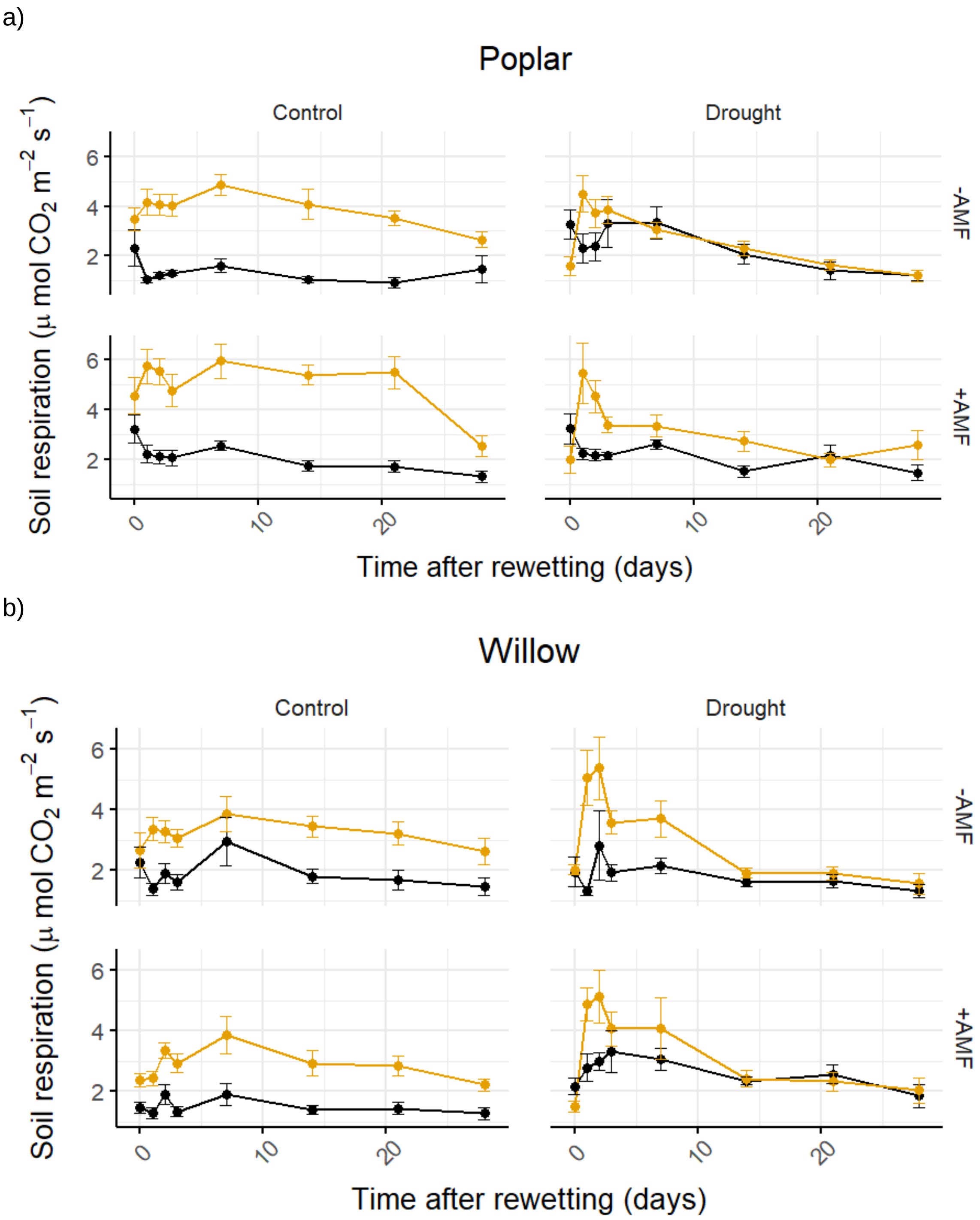


Figure 3.5 *Soil respiration under a) poplar b) willow following a drought-rewetting event (Drought) or established watering regimen (Control), inoculated with arbuscular mycorrhizal fungi (+AMF) or without fungal inoculation (-AMF) in soils amended with nitrogen (yellow) compared to unfertilised soils (black); respiration rates are shown from the day of rewetting until the end of the experiment 28 days later; dots and*

whiskers represent means and standard errors for n=8, where n is the number of replicates per treatment combination per tree species,.

Nutrient availability

Nitrogen exchange rates (total inorganic N, ammonium-N, and nitrate-N) were below detection limits in most of the pots and were therefore not tested statistically. Of the 128 pots that received ion exchange resins, nitrate-N exchange rates were above detection limits in only 48 pots, spread evenly across species and treatments. Ammonium-N rates were above detection limits in just four pots (two willow and two poplar), three of which were inoculated with AMF and were fertilised with P (data not shown).

Phosphorus exchange rates in the soil were influenced by N- and P-addition in both species (poplar: $F_{2,62} = 60.8$, $p < 0.001$ and willow: $F_{2,62} = 21.8$, $p < 0.001$) but not by drought or AMF inoculation. Unsurprisingly, soil P exchange rates were higher in pots with added P compared to pots without P ($p < 0.001$ for both species) and were lower in pots with added N ($p < 0.001$ for both species; Appendix B6).

Potassium exchange rates did not differ between drought or AMF inoculation treatments, but the effects of fertilization differed between species. Under poplar, soil K exchange rates were significantly higher in pots with added P than pots without P ($p = 0.0012$) and tended to be higher with N-addition compared to pots without added N ($p = 0.059$). However, N-addition reduced the effect of P-addition on K exchange rates (N × P interaction: $p < 0.001$; Figure 3.6, Appendix B6). Hence, the final model for K exchange rates under poplar included the interaction between N- and P-addition ($F_{4,59} = 16.0$, $p < 0.001$). Under willow, K exchange rates were higher with N-addition ($p = 0.0053$) and P-addition ($p = 0.0048$) but there was no interaction between N and P addition ($F_{2,61} = 8.22$, $p < 0.001$; Figure 3.6, Appendix B6).

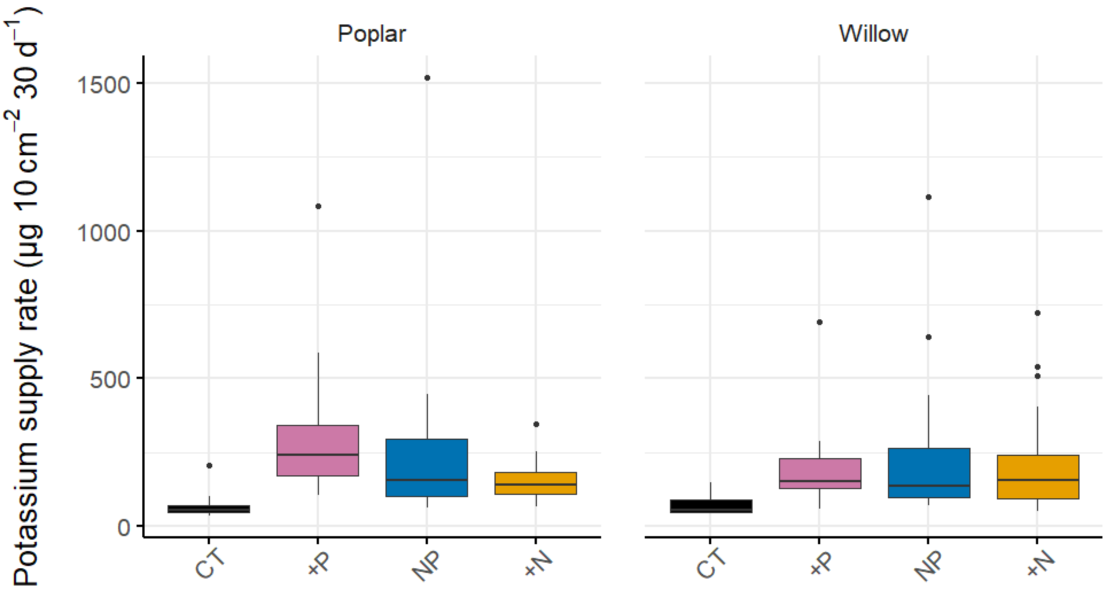
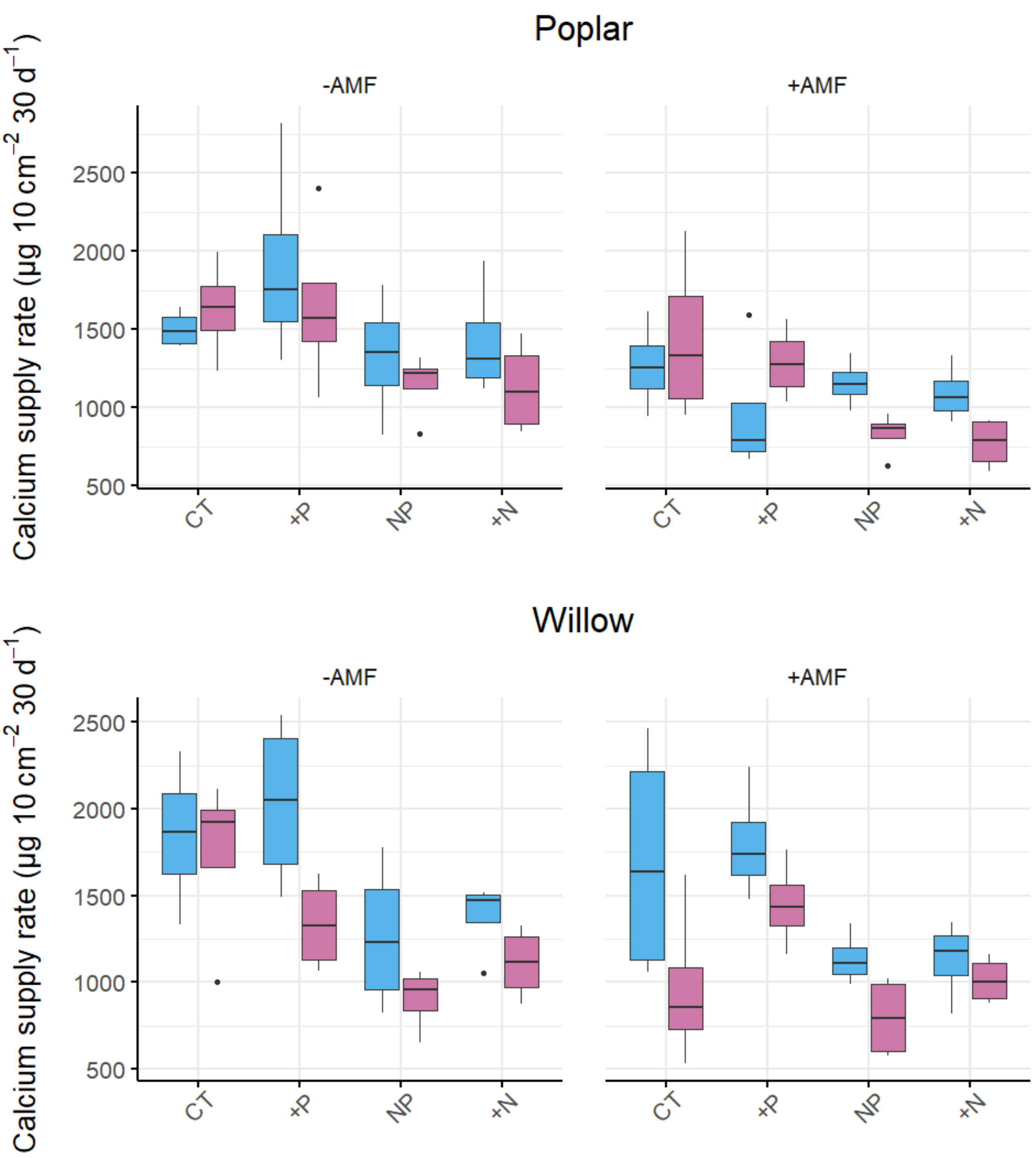


Figure 3.6 *Potassium exchange rates in soils under Poplar and Willow trees amended with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+NP) compared to unfertilised soils (CT) during days 14-28 after imposing rewetting in the drought /rewetting treatment; boxes denote the 25th and 75th percentiles and median lines are given for n = 16, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5× the interquartile range, and dots indicate outliers.*

Calcium exchange rates were affected by drought, N-addition, and AMF inoculation in both species. Under willow, Ca exchange rates were significantly lower in pots with added N compared to pots without N ($p < 0.001$), in droughted compared to un-droughted pots ($p < 0.001$) and in AMF-inoculated pots compared to uninoculated pots ($p = 0.027$; Figure 3.7a, Appendix B6). The final model therefore included drought, AMF inoculation and N-addition, but no interaction terms ($F_{3,60} = 18.75$, $p < 0.001$). Under poplar, the model for soil Ca exchange rates included AMF inoculation and the interaction between N-addition and drought ($F_{5,58} = 8.04$, $p < 0.001$), whereby Ca exchange rates were lower in pots inoculated with AMF compared to uninoculated pots ($p < 0.001$) and lower in droughted pots with N-addition compared to droughted pots without N ($p = 0.034$, Figure 3.7a; Appendix B6).

Magnesium exchange rates were affected by interactions between drought and nutrient additions in both species. In poplar, there was no overall effect of drought, AMF inoculation or either nutrient addition treatment but Mg exchange rates were lower in droughted pots with N-addition compared to droughted pots without N ($F_{3,60} = 5.59$, $p = 0.0018$; N × drought interaction; Figure 3.7b; Appendix B6). In willow, the final model for Mg exchange revealed no overall effect of any single treatment, but a significant four-way interaction between drought, AMF inoculation, N-addition, and P-addition ($F_{15,48} = 3.17$, $p = 0.0012$). Drought appeared to reduce Mg exchange rates depending on nutrient addition and AMF inoculation: In uninoculated pots, Mg exchange rates were lower with drought and N or P-addition, but in AMF-inoculated pots Mg exchange rates were only lower with drought and combined N and P-addition (Figure 3.7b; Appendix B6).

a)


Poplar
-AMF
+AMF
Calcium supply rate (µg 10 cm^{-2} 30 d^{-1})
2500
2000
1500
1000
500
CT
+P
NP
+N
Willow
-AMF
+AMF
Calcium supply rate (µg 10 cm^{-2} 30 d^{-1})
2500
2000
1500
1000
500
CT
+P
NP
+N

b)

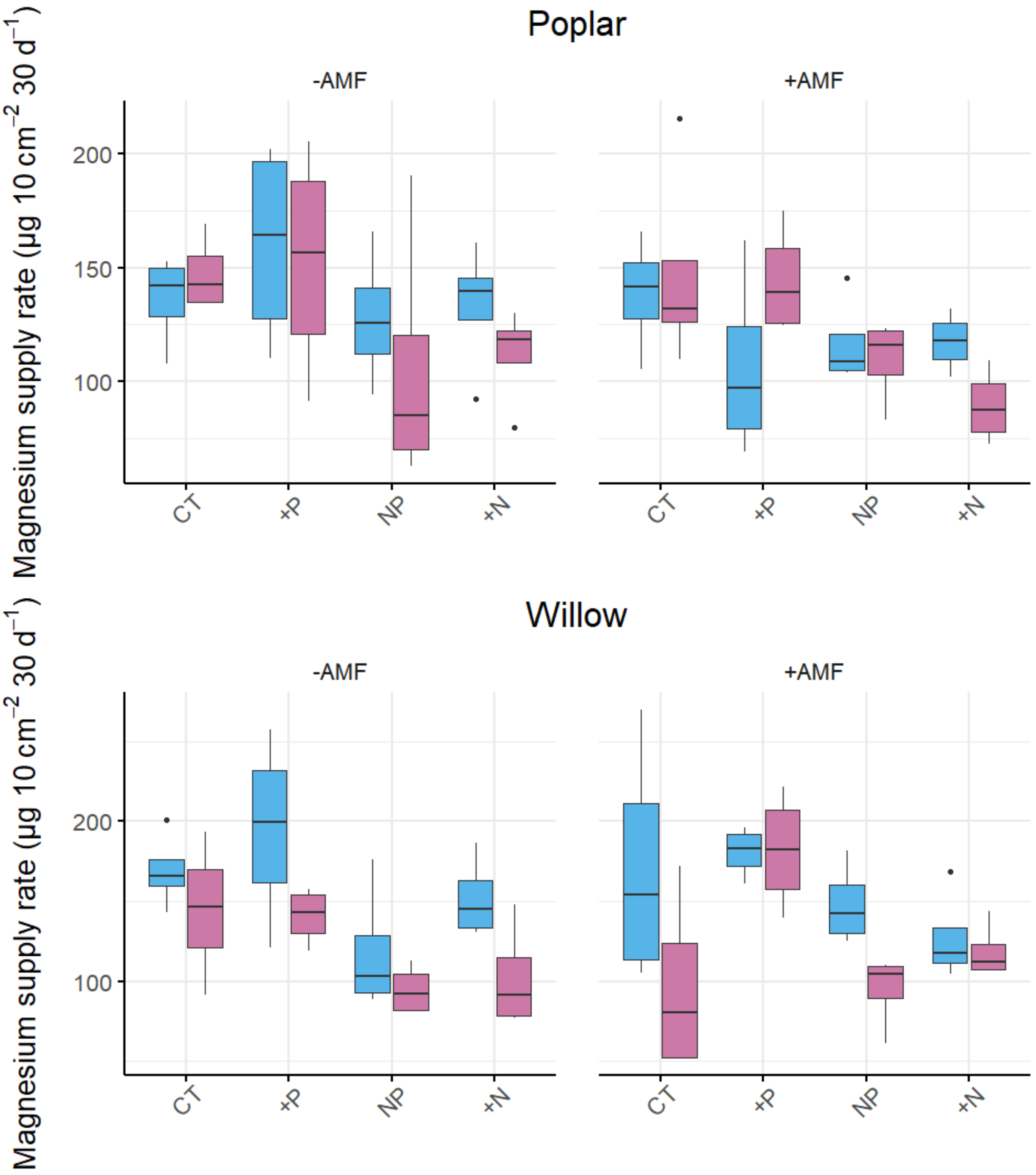


***Figure 3.7** Exchange rates for a) Calcium, b) Magnesium in soils under poplar and willow trees inoculated with arbuscular mycorrhizal fungi (+AMF) or without fungal inoculation (-AMF) in soils amended with nitrogen (+N), phosphorus (+P), nitrogen and phosphorus (+NP) compared to unfertilised soils (CT) following a drought-rewetting event (pink) or control watering regimen (blue); boxes denote the 25th and 75th percentiles and median lines are given for n = 4, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5× the interquartile range, and dots indicate outliers.*

3.4 Discussion

My study demonstrated that the interactions between N-addition and AMF inoculation of SRC crop species subjected to DRW can influence soil carbon dynamics upon rewetting. Importantly, CO_2 release through Birch effects upon rewetting was greater in soils with added N, but soil respiration in the following 28 days varied between species and depended on the interaction between N-addition and AMF inoculation. Thus, my study demonstrates how species identity, AMF inoculation, fertilization and drought all interact to influence nutrient exchange rates in the soil. Here, I interpret my findings within the context of bioenergy crop management and discuss the wider implications for carbon dynamics in these crops.

Peak soil CO_2 release by Birch effects

In partial support of my first hypothesis, my study highlighted that rapid rewetting produced a pulse of soil CO_2 upon rewetting (Birch, 1958). However, in contrast to expectations, the respiration pulse upon rewetting was considerably larger with N-addition (Figure 3.5a; Figure 3.5b). This is at odds with previous studies demonstrating a reduction in soil respiration in forests under N-addition (Janssens et al., 2010; Sun et al., 2014; Zhou et al., 2014). Studies of DRW events also highlight a substantial release of N during rewetting, which is attributed to the mineralization of N in soil organic matter (Birch, 1958; Canarini and Dijkstra, 2015; Rodríguez et al., 2019; Erinle, Bengtson and Marschner, 2021; Shen et al., 2022). Although there are few studies of the effects of nutrient addition on the magnitude of Birch effects, I postulated that N-addition should reduce the magnitude of Birch effects by providing additional N for microbial use, thereby reducing the need for microbial mining of N (Zhao et al., 2022). Although the magnitude of the Birch effect was greater with N-addition in soils under both species, the peak was short-lived and by day three, respiration rates had stabilised (Figure 3.5a; Figure 3.5b). Hence, while N-addition to soil makes soil carbon dynamics more susceptible to intense drying and rewetting events, the impact is transient (Erinle, Bengtson and Marschner, 2021)

The substantial release of CO_2 from the N-fertilized soils upon rewetting can be attributed to two mechanisms: First, greater growth and biomass of the trees with N-addition (Chapter 2) would also increase plant water requirements, resulting in a more severe drought in N-fertilized pots; previous work has shown that Birch effects are generally higher under more severe drought (Xu and Luo, 2012). Thus, although N-addition can offer a short-term mitigation of water deficiency stress in plants (Song et al., 2019; Wang et al., 2020), the greater biomass, and therefore water demand, of N-fertilized trees in my study may have made them more susceptible to drought. Second, N-fertilized trees had greater root biomass (Chapter 2), and decay of fine roots that died during the drought could contribute to the large Birch effects upon rewetting. Nonetheless, my study clearly highlights that any increase in soil respiration upon rewetting is short-lived and when the trees are supplemented with N-addition within recommended guidelines, post-drought soil respiration is reduced, suggesting that short, intense pulses of CO_2 upon rewetting would not increase the total amount of carbon released to the atmosphere through soil respiration.

It is noteworthy that neither the magnitude nor the timing of CO_2 release upon rewetting changed with AMF inoculation, indicating that AMF respiration and turnover have little influence in the occurrence of Birch effects in this study system (Figure 3.5a; Figure 3.5b). Previous studies of soil microbial communities have shown that respiration rates from microbial communities exposed to DRW events contribute to peak CO_2 efflux after re-wetting but that overall respiration remains lower than controls long after the drought treatment has ended (Chowdhury, Burns and Marschner, 2011). The structure of the AMF community may also be important, as different species may have distinct tolerances to DRW events, depending on e.g. the dormancy periods of spores, germination speed after rewetting, and the extent of mycelial breakdown (Braunberger, Abbott and Robson, 1996). In addition, there is evidence that plants may be partly responsible for the lack of an AMF respiration response to rewetting because DRW events can drive increased uptake of nitrogen by plants to support their own recovery at the expense of N uptake by microbial communities (Dannenmann et al., 2009). However, although there is clear evidence that microbial communities are impacted by drought independently of plants, the responses of AMF to DRW events remain largely uncharacterised.

Post-rewetting soil respiration

In partial support of my second hypothesis, soil respiration was notably higher during the month following rewetting in willow trees with AMF inoculation and N-addition compared with control pots. However, N-addition reduced the effect of AMF inoculation (Figure 3.5a; Figure 3.5b). The higher soil respiration under willow with N-addition contradicts studies in temperate forests indicating a reduction in soil respiration with N-addition (Camenzind et al., 2016; Bowden et al., 2019). Despite the large pulse of CO_2 upon rewetting, neither AMF inoculation nor N-addition had significant effects on soil respiration under poplar during the month after rewetting, although AMF inoculation interacted with N-addition to influence respiration rates in the month after the drought. The most prevalent limiting factors in the survival of SRC crops are water and nutrient availability (Åhman and Larsson, 1994). Drought can be a stronger driver than N-addition in changes to soil respiration (Wang et al., 2014b; 2021b; Nguyen et al., 2018b; Preece, Farré-Armengol and Peñuelas, 2020). Numerous studies in natural and agricultural ecosystems have shown that association with AMF can buffer the impacts of drought on plants (Braunberger, Abbott and Robson, 1996; Aroca, Porcel and Ruiz-Lozano, 2007; Kivlin, Emery and Rudgers, 2013; Wu, Srivastava and Zou, 2013; Li et al., 2014; Kilpeläinen et al., 2017). The rapid recovery of respiration rates in soils inoculated with AMF could be due to the important role of glomalin (a glycoprotein) in soil structure.

Glomalin is produced by AMF hyphae and has hydrophobic properties (Wright and Upadhyaya, 1998), which contributes greatly to the formation of soil aggregates and promotes resistance to changing soil water availability (Wright and Nichols, 2002). Glomalin is abundant in soils with large AMF hyphal networks, accounting for *c*. 30% of soil carbon in AMF-dominated systems (Wright and Nichols, 2002). Soil fungi in general are thought to be resistant to short-term drought (Kivlin, Emery and Rudgers, 2013) and soil fungal communities under SRC crops are often highly diverse (Vanbeveren and Ceulemans, 2019), which could aid in overall resilience of soil carbon dynamics to intense moisture changes. Interestingly, the stimulatory effect of AMF on soil respiration appeared to be weakened by N-addition. Previous work has also showed that AMF abundance declines under N-addition (Camenzind et al., 2016) but I was unable to assess differences in AMF colonisation among fertilisation treatments. It is possible that the addition of N in this study reduced the requirement of AMF to expand hyphal networks, which would slow mycelial turnover and therefore reduce soil respiration (Wang et al., 2021b). It is also possible that AMF inoculation increased soil respiration indirectly via root respiration. In trees inoculated with AMF, root respiration can increase by up to 40%. depending on both the plant species and the AMF community (Fahey et al., 2016; Yue et al., 2021). In addition, it is important to note that both plant and AMF respiration can also be altered by changes in soil moisture content (Yue et al., 2021).

Relationships between plants and AMF could explain the differences in post-drought soil respiration between willow and poplar. AMF communities are often nested, as they originate through early coloniser species and evolve into more specialist communities (van der Heijden et al., 2015b). Early AMF colonisers are often generalist species, which can form mutualistic relationships with many plant species, whereas specialised AMF species have developed to form relationships with specific sets of plants and abiotic conditions as the ecosystem evolves (Smith and Read, 2008b). The associations between host plants and AMF shift during the growing season, highlighting the important of role abiotic conditions in the development of AMF communities (Bennett et al., 2013). In my study, a slow decline of generalist AMF communities with a concomitant increase of more specialised AMF species in soils under willow than under poplar (van der Heijden et al., 2015b) could contribute to both the differences between species and the increased respiration rate in the month following rewetting in AMF inoculated pots. Alternatively, the increase in respiration could have been a direct result of the changing abiotic conditions which led to a rapid change in the AMF community (Bennett et al., 2013), as well as growth and increased activity of other soil microorganisms. As respiration by roots, AMF, and other soil microorganisms all contribute to soil CO_2 efflux, and all of these components can be influenced by DRW events and fertilization, partitioning studies will be required to determine the relative contributions of plants, their AMF partners and the wider soil microbial community to changes in soil respiration after drought.

There was a marked difference between tree species in the interactive effects of AMF and N during post-drought recovery (Figure 3.5a; Figure 3.5b). Soil respiration immediately after the drought was strongly depressed in poplar trees with N-addition but, in contrast to willow, neither N nor AMF had much influence on post-drought respiration rates beyond the first few days after rewetting. Although both tree species have high water requirements, willow is thought to be particularly suited as a SRC crop species for soils prone to flooding (Dimitriou, Busch and Jacobs, 2009). I therefore expected greater effects of drought on soil carbon dynamics under willow. However, the drought had a greater and longer lasting effect on soil respiration under poplar, and neither N-addition nor AMF mitigated the legacy effects of drought on soil respiration under poplar during the subsequent weeks. Hence, despite the high-water requirements of willow, soil carbon dynamics are more likely to be impacted by frequent drought under poplar SRC crops.

Nutrient availability

The findings of this study only partially support my final hypothesis, as the DRW event generally reduced the availability of nutrients. However, as hypothesised, the effects of drought, nutrient addition and AMF inoculation on nutrient exchange rates varied greatly between tree species. Although the exchange rates of Ca, and Mg were impacted by drought and N-addition under both species, my results showed that AMF inoculation generally had a greater impact on nutrient availability in soils under willow than under poplar.

Overall, declines in nutrient exchange rates in soils with added N likely indicate greater nutrient uptake to support the additional growth and biomass of N-fertilized trees (Chapter 2). Higher K exchange rates in soils with added N or P compared to controls are a direct result of the K added with the fertilizer treatments (Figure 3.6). However, as all nutrient addition treatments included equal amounts of K, the lower K exchange rates under poplar with added N compared to added P also indicate greater K uptake by N-fertilized trees (Figure 3.6). As nutrient additions were made once at the beginning of the growing season in June 2020 and drought then rewetting was not implemented until August 2020 it is unlikely that rewetting resulted in substantial loss of mobile nutrient elements such as K. In addition, there was no evidence of leaching by water draining from the bottom of the pots. Instead, as both willow and poplar have a high N and water requirement, especially during the growing season, rapid uptake of available nutrients before rewetting is very likely (Weih and Nordh, 2002; Dimitriou and Mola-Yudego, 2017;

Seo et al., 2017), especially as the very low concentrations of available N suggest that the high growth rates of these SRC crop plants rapidly depleted the finite mineral resources in the pots.

Calcium is rarely deficient in most soils but Ca exchange was reduced under both tree species when inoculated with AMF, suggesting that AMF associations might improve plant Ca uptake, which has previously been demonstrated for acidic soils (Clark and Zeto, 2000). Calcium concentrations in leaf biomass of crops can increase with AMF hyphal abundance in the soil (Liu et al., 2002). Importantly, Ca is vital to woody regrowth after dormancy periods (Navazio and Mariani, 2008) and although there are no recommendations for Ca fertilization of SRC crops, increased Ca uptake in AMF inoculated trees could aid regeneration after coppicing. However, Ca exchange rates appear to decrease with N-addition (Ai et al., 2017), as shown in my findings (Figure 3.7a). Calcium exchange rates were also lower in droughted vs. un-droughted soils under both species, which may reflect greater Ca uptake to improve water use efficiency in woody plants (Li et al., 2021).

My findings for Mg exchange rates highlight the importance of understanding the interactions between nutrient addition and changes in abiotic conditions. Overall Mg exchange rates were not altered by individual treatments in either species but the interactions between nutrient addition treatments and drought generally reduced Mg exchange rates in both species (Figure 3.7b). Although no previous experiments to my knowledge have assessed the combined influence of fertilization and DRW events on nutrient availability, some studies have shown that either N-addition or drought can reduce Mg availability (Wikstrom and Ericsson, 1995; Ai et al., 2017), indicating that an interaction between N and drought would likely exacerbate the decrease in Mg exchange rates. The role of AMF in lowering Mg exchange rates under willow is difficult to unpick, but AMF can help mediate Mg deficiency in soils (Zhang et al., 2015) and inoculation of plants with AMF is likely to aid the uptake of nutrients such as Mg even in depleted soils (Liu et al., 2002). Magnesium is a critical nutrient in the production of chlorophyll, impacting both above- and belowground biomass (Kölling et al., 1997) and soils may become Mg-deficient when exposed to intense DRW cycles (Wikstrom and Ericsson, 1995; Kölling et al., 1997). Willow is a high-yield plant and young plantations of willow are likely to reach higher yields than young plantations of poplar (Ceulemans, McDonald and Pereira, 1996). It is therefore possible that the lower Mg exchange rates under AMF-inoculated willow trees are the result of high Mg requirements and efficiency of the AMF community under willow in facilitating Mg uptake. Thus, although there are no recommendations to consider Mg in fertilisation plans for SRC crops, my results indicate greater Mg requirements for fertilized SRC crops under drought, and the potential for AMF to aid in plant Mg uptake.

My findings add to the growing body of literature showing that drying and rewetting of soils can reduce nutrient availability even after soil moisture has returned to a normal level (Zhang, Wang and Li, 2022). However, lower exchange rates of Ca and Mg in my mesocosms likely indicate greater plant uptake to mitigate the impacts of drought, which was also influenced by AMF inoculation. Hence, drought may create legacy effects on plant–soil nutrient dynamics (Oliver, Finch and Taylor, 2009; Tripathi et al., 2018), altering fertilization requirements in SRC crops.

3.5 Conclusions

To my knowledge, my study is the first to assess how nutrient addition and AMF inoculation influence DRW events in SRC crop species. Understanding how nutrient availability and mycorrhizal associations might influence plant-soil carbon dynamics in bioenergy crops is essential to determine how changing water availability could affect soil carbon storage and release under increasing frequency of extreme weather events.

My findings demonstrate that enhanced growth and greater biomass of trees with N-addition could exacerbate the effects of drought on soil carbon and nutrient dynamics, and result in greater release of CO_2 through Birch effects upon rewetting. Partitioning studies should investigate the relative contributions of roots, AMF, and broader soil microbial communities to soil respiration during different phases of DRW events. In particular, the role of AMF in mitigating the impacts of drought requires further work, but my results suggest that AMF inoculation could mitigate some effects of drought stress by improving plant nutrient uptake. Overall, my work sheds light on the potential impacts of drought on carbon and nutrient cycling under SRC crops. The potential for AMF inoculation or fertilization with Ca and Mg to mitigate drought stress in SRC crops is worthy of further attention. Given that drought events are likely to become more frequent and more severe, future studies should focus on the impact of repeated DRW cycles and the effect of drought intensity on CO_2 release through Birch effects.

4. Interactive effects of flooding, N-addition, and drought history on growth and carbon dynamics of willow and poplar

4.1 Introduction

Global climate change is influencing precipitation rates by moving storm events from the equator towards the poles and intensifying existing precipitation events (Marvel and Bonfils, 2013). Global annual precipitation increased by 2 mm per decade between 1901 – 2011 (Blude and Arndt, 2016) and future climate scenarios show the intensification of precipitation events and extreme flooding (Daliakopoulos et al., 2016; Oppenheimer et al., 2019; Gould et al., 2020). Most of the areas at risk comprise dense human populations with substantial areas of low-lying fertile agricultural land (Gould et al., 2020). Indeed, agricultural systems in low-lying areas with temperate climate such as the UK are most at risk of the increasing frequency and intensification of flooding (Gould et al., 2020) and loss of fertile soils (Oppenheimer et al., 2019). Much of this land is in the North East, where large areas of fertile arable soils are found, growing a variety of geographical and socio-economically important crops such as potatoes, winter cereals and grasses (Gould et al., 2020). Flooding events affect productivity of economically important arable land in temperate climates (Rutto, Mizutani and Kadoya, 2002; Chen et al., 2019; Bi et al., 2020; Jonathan et al., 2020), and this is due, in part, to soil erosion and nutrient leaching into nearby watercourses (Kaur et al., 2020). The use of flood-tolerant species such as willow and poplar as a strategy to mitigate the impact of flooding on soils has been well studied (Vidon, Welsh and Hassanzadeh, 2019). These flood-tolerant plants respond to high moisture conditions by releasing organic compounds into the rhizosphere, providing the microbial community with substrate (Barber and Martin, 1976), while facilitating improved soil properties such as soil porosity through widespread root networks (Kahle and Janssen, 2020).

Willow and poplar are also used as short rotation coppice (SRC) crops in many countries with temperate climates, including the UK. SRC crops provide a carbon-neutral energy source as an alternative to fossil fuels, and the UK government predicts that at least 14% of national energy needs will be met from SRC willow and poplar crops (Department for Environment Food & Rural Affairs, 2020). The status of SRC crops as a less carbon intensive energy source (Verlinden et al., 2013) and their potential to sequester high amounts of carbon is of substantial interest to policymakers in agriculture, energy, and climate sectors (HM Government, 2011). Importantly, rapid young growth is a mitigation strategy to overcome adverse abiotic conditions such as periodic flooding (Hartwich, Bölscher and Schulte, 2014). To facilitate rapid growth, SRC crop species create a well-developed root network, which also allows rapid uptake of large amounts of water, thus reducing surrounding soil moisture levels (Hartwich, Bölscher and Schulte, 2014). Because of their rapid early growth, SRC crops have a large carbon sequestration capacity, most of which is stored within the woody stem biomass. In poplar SRC crops, the aboveground woody biomass contains c. 80% of the total biomass C (Schoeneberger et al., 2012) and in willow, the total aboveground biomass (including leaves) also accounts for c. 80% of total biomass carbon (Rytter, Rytter and Högbom, 2015). Soils under SRC crops also generally have increased carbon stocks, with many studies showing that fast-growing biomass (Rewald, Kunze and Godbold, 2016), large root systems (Phillips, Marden and Suzanne, 2014), minimal nutrient input (Tubby and Armstrong, 2002) and no disturbance of the understorey or soil for several years (Kahle and Janssen, 2020) all improve soil carbon capture (Rytter, Rytter and Högbom, 2015).

SRC species such as willow and poplar are routinely recommended for planting in riparian zones to control nutrient leaching from agricultural soils into surrounding watercourses (Vidon, Welsh and Hassanzadeh, 2019). Willow and poplar are very effective at reducing nitrogen leaching, as they establish and grow rapidly and are able to take up large amounts of nitrogen to aid growth (Aronsson, Bergström and Elowson, 2000; Dimitriou and Aronsson, 2004). Despite this, there are few studies focusing on how SRC crops alter availability of nutrients in soils, and even fewer that also consider the impacts of changing abiotic conditions such as flooding, which flushes nutrients and pollutants through soils. Nonetheless, one study showed that increases in heavy metal concentrations, such as zinc, after flooding events were smaller in soils under SRC than under other arable crops, possibly due to increased decomposition often found under established SRC crops (Zimmer et al., 2012) or because SRC crop species such as willow and poplar have a high propensity for accumulating heavy metals, which makes them well suited to bioremediation of soils (Riddell-Black, 1994; Laureysens et al., 2004). Less is known about how intensive flooding events will affect the yield and carbon storage capacity of these species when grown as dedicated bioenergy crops (Sevel et al., 2014a). Given that SRC species such as willow and poplar are often found growing around natural bodies of water such as river floodplains, and their planting is widely advised on flood-prone soils (Fischer et al., 2018), understanding the impacts of intensive flood events on the carbon balance of SRC crops is crucial. Studies that have focused on SRC species specifically in flood-prone areas have shown that the highly variable water content in soils under threat of extreme flood events can create a hotspot for carbon cycling (Yuan et al., 2013). The interactions between land use and soil moisture saturation have substantial impact on soil respiration, whereby flooding increases CO_2 efflux especially in soils which are conventionally managed for arable crops (Zhu et al., 2020). By contrast, in riparian zones planted with SRC species, net CO_2 emissions were ~1.4 times lower than non-flooded areas because high soil moisture content created anaerobic conditions (Silverthorn and Richardson, 2021). However, water-saturation of soils can also create areas with high soil emissions of other greenhouse gasses (GHGs), such as methane (CH_4).

Agriculture is responsible for 47% of total CH_4 emissions in the UK (Department for Business, Energy & Industry, 2021) and global CH_4 emissions have increased by over 50% in the last 30 years, making it the second most significant GHG behind CO_2 (Department for Business, Energy & Industry, 2021). Methane emissions from agricultural soils occur when soils become water-saturated as a result of poor drainage and intensive flood events, contributing to anaerobic conditions favourable to methanogenic microbes (Kern et al., 2012). In temperate soils, CH_4 emissions are much higher in organic soils with high water tables, which tend to be poorly draining, which suggests that using SRC crops to reduce soil moisture levels has the potential to reduce CH_4 emissions (Don et al., 2012; Richards et al., 2017). Soil moisture is a key component of whether a soil is a sink or source for CH_4, with as little as a 1% increase in soil water content creating a noticeable increase in CH_4 emissions (Kern et al., 2012). Soil moisture is thus the key factor controlling CH_4 emissions in SRC crops (Silverthorn and Richardson, 2021) and there is a vital need to understand how flooding will affect soil GHG emissions under SRC crops, especially when environmental benefits such as combatting soil degradation, stabilising soil carbon stocks and reducing GHG emissions in both the agricultural and energy sectors, are among the main incentives for SRC crop establishment (Rowe, Street and Taylor, 2009; Lockwell, Guidi and Labrecque, 2012; Ferré and Comolli, 2018). As SRC crops can be grown on nutrient-poor marginal land with highly variable water supply (Dimitriou, Busch and Jacobs, 2009), it is also essential to establish how land management and interacting extreme weather events affect SRC crops (Vidon, Welsh and Hassanzadeh, 2019) and soil GHG fluxes. This might be particularly important for SRC crops in the UK, because global change is predicted to cause wetter winters and hotter summers, with frequent periods of heavy rainfall events or drought (Met Office, 2021).

While drought and flood events are widely studied, their effects are often investigated separately. However, drought and flood events are opposite extremes of the same climatic processes and often occur in succession (He and Sheffield, 2020). While the effects of an individual drought or flood event may not be extreme, when one follows the other the compound effects often exacerbate the individual effects (Rezvani, Na and Najafi, 2023). Where drought has altered soil structure and vegetation and is then followed in close succession by flooding, which causes increased waterflow and the loss of soil and nutrients, the impacts of both the drought and flood are intensified to extreme conditions (He and Sheffield, 2020). Importantly, these compound events are occurring at higher intensity and frequency, thus creating more extreme abiotic conditions as the climate continues to change (Rezvani, Na and Najafi, 2023). Although the climate mechanisms leading to extreme weather events such as drought or flood are complex and the underlying causes of compound events are difficult to unpack (He and Sheffield, 2020), compound events are now recognised as important phenomena to study. Drought can have a stronger legacy effect than flooding, due to poor recovery by plants and the damaging effects of prolonged drought on soil microbial communities (Nguyen et al., 2018a). However, flooding also reduces the growth and health of crops (Kaur et al., 2020), while causing immediate substantial increases in soil CH_4 emissions (Sánchez-Rodríguez et al., 2019).

Fertilization of SRC crops could interact with extreme weather events such as floods to boost GHG emissions from soils. Previous work has established that N-addition boosts soil CO_2 emissions after flooding by enhancing biological activity in the soil and increasing plant biomass (Tang et al., 2018). The processes controlling CH_4 emissions are also impacted by N-availability because methanogenic microorganisms appear to have high N requirements, and thus their population size and activity increase when supplied with N fertilizers (Yao et al., 2012). Organic carbon sources are required for CH_4 production and as enhanced plant growth with N-addition increases carbon inputs to the soil (Schimel, 2000), which increases methanogenic populations and CH_4 production (Cai, Shan and Xu, 2007). However, we do not know how fertilization will interact with flooding to affect soil GHG fluxes from SRC crops. Filling this knowledge gap is important, because SRC crops are recommended for marginal land, where flood risk can be high (McKay, 2011; Schweier and Becker, 2013) but the need for nutrient additions to crops might also be greater (Caslin, Finnan and McCracken, 2010; Dimitriou and Mola-Yudego, 2017; Georgiadis et al., 2017). Studies investigating the effects of N-addition on CH_4 on rice crops show that the interactions between flooding and N-fertilization are not clearly understood, as N-addition has variably produced an increase, decrease and no effect on CH_4 fluxes (Minami, 1995). However, the use of N fertilizers to combat poor soil fertility in flood-prone areas may not have the same magnitude of effect when these areas have a drought history (Nguyen et al., 2018a) and GHG emissions during flood events could increase in soils with a history of drought (Liu et al., 2022). Such interactions between shifting abiotic conditions and fertilization of SRC crops are likely to affect their environmental benefits (Davis et al., 2019; Silverthorn and Richardson, 2021).

Consequently, knowledge of how fertilization and variable soil moisture conditions might influence carbon dynamics and GHG emissions before, during, and after a flood event can inform the establishment and management of SRC on marginal land.

In this study I assess how fertilisation and drought history influence soil CO_2 and CH_4 emissions and nutrient availability under established SRC crop species willow (*Salix purpurea*) and poplar (*Populus nigra*) subjected to flooding. I grew willow and poplar saplings in mesocosms with added N or P for one year before subjecting half of the trees to a strong drought event (Chapter 3). In the following spring, half of the trees (from control and drought treatments) were subjected to a 14-day flood event. I measured soil CO_2 and CH_4 efflux and soil nutrient availability before undertaking a full destructive harvest to measure plant growth and biomass to test the following hypotheses:

H1) Flooding will increase soil CO_2 and CH_4 emissions under SRC crops and the increase will be exacerbated by N-addition.

H2) Drought history will reduce CO_2 and CH_4 emissions under SRC crop species.

H3) Flood and drought history will reduce the availability of soil nutrients.

H4) Tree growth and biomass will be reduced in mesocosms exposed to drought, flood or both extreme weather events, but the reduction will be offset by N-addition, whereby N-fertilised trees will have higher foliar N concentrations, growth rates and biomass compared to trees without N, irrespective of extreme weather events.

4.2 Methods and materials

Experimental design

To establish how a flood event influences plant–soil carbon dynamics, including CO_2 and CH_4 emissions, in SRC crops I used a sub-sample of an established large pot study (Chapter 2 & 3). I grew *Populus nigra* L. (poplar) and *Salix purpurea* L. (willow), which are both common bioenergy crops in temperate climates such as the UK and are often planted on marginal land such as degraded agricultural soils and areas at risk of flood. In May 2019, I set up 192 18 L pots (30-cm diameter, 30-cm height) in two polytunnels at the James Hutton Institute, Dundee, Scotland. Each polytunnel was split into three blocks per species (six replicate blocks per species in total), and 16 pots were assigned to each block. Each pot was filled with 17.5 L of pre-sterilised sandy loam soil, made from a 1:1 mix of commercial loam (Keith Singletons, Cumbria, UK) and sand, which had been autoclaved for 2 h. Half of the pots per block were inoculated with a commercial AM fungal inoculant (AMF+), whereby each pot was layered with 5 L sterilised soil, followed by 7.5 L soil mixed with 50 g rootgrow^{TM} granules (PlantWorks

Ltd, Kent, UK), and then another 5 L of sterilised soil. The other half were left as uninoculated controls (AMF-).

I grew poplar (The Poplar Tree Company, Madley, UK) and willow (The Willowbank, Lydbrook, UK) from 30-cm cuttings. I planted 100 cuttings of each species in Deepot^{TM} cells (6-cm diameter, 25-cm depth; Stuewe & Sons Inc, Oregon, USA) with 650 ml autoclaved sandy loam. After four weeks of growth, 96 randomly selected cuttings per species were transferred to 18 L pots (16 pots per species per replicated block). Cuttings were removed from the Deepots^{TM}, the root ball gently rinsed with cold water, and then half the cuttings were planted in the +AMF pots, half in the -AMF pots. The cuttings were planted to one side of the pot to allow space for soil measurements and all cuttings were pruned to ensure only the main stem was left. Each pot was watered automatically twice daily at 8.30 am and 3.30 pm for 90 seconds, using two pressure-compensated spray stakes (flow rate 0.2 L min-1; Netafim Ltd. Skelmersdale, UK). Spray stakes were located on opposite sides of each pot, with the spray directed towards the centre.

Fertilization levels were based on recommendations for bioenergy crop establishment, whereby 120 kg N ha^{-1} yr^{-1} and 80 kg P ha^{-1} yr^{-1} are considered a high level of fertiliser (Tubby and Armstrong, 2002; Sevel et al., 2014b; 2014a; Georgiadis et al., 2017). To ensure the correct amounts of N and P were added to each treatment, I created a stock solution using different chemical compounds for each treatment. The +N pots each received 8.46 g KNO_3 and 2.21 g NH_4NO_3. The +P pots each received 7.28 g K_2HPO_4, and the +NP pots received 5.67 g of NH_4NO_3 and 7.28 g of K_2HPO_4. Hence, each of the three fertilization treatments also had added potassium (K) at a rate of 202 mg K L^{-1} yr^{-1}. The trees were fertilised in a single application in June 2020, to mimic conventional annual fertilizer application to bioenergy crops in the field.

To assess how SRC crops recover from a flood event and establish how this alters soil CO_2 and CH_4 emissions, as well as soil nutrient availability, tree growth, biomass, and foliar N, I created a flooding event using four blocks per tree species (two from each polytunnel) between April 2021 and May 2021. Half of the trees used in the experiment had been subjected to a drought treatment during the previous growing season (Chapter 3). At the beginning of the experiment, the trees were well established, fully recovered from any visible signs of distress caused during the drought and had emerged healthy from winter dormancy. Half of the pots in each block were randomly assigned flood and half continued with the normal watering regime. To assess the possible effects of drought history, I assigned the flood treatment equally to both control and previously droughted pots. As AMF inoculation only had a slight influence on soil respiration during the previous drying-rewetting event, and effects were most substantial in N addition pots

(Chapter 3), I assigned the flooding treatment creating factorial conditions: flood or no flood, added N or without N, drought history or no drought history. Hence, the AMF inoculation treatments were spread equally over flooded and previously droughted treatments but were confounded with combined treatments. For example, all flooded pots without N-addition were uninoculated, whereas all flooded pots with N-additions were AMF-inoculated. The AMF treatments are therefore not considered in this experiment. In total, there were eight replicates per species and treatment for a total of eight treatments (two N-addition treatments x two flood treatments x two historic drought treatments), equalling 128 pots. In April 2021, a flexi-tub was placed under each pot assigned a flood treatment and a flood treatment was imposed by watering the pots until the soil was saturated. Each flexi-tub was filled with water until the soil in the pots was waterlogged, whereby each pot was fully submerged in water ensuring the soil was saturated. Water levels were monitored daily to ensure levels did not fall below field capacity and the flexi-tubs prevented drainage. Although few studies focus on CH_4 efflux of flooded soils under temperate tree species, 14 day flood periods have previously been used to simulate intense flood events often found in these biomes (Khalid, Shaaban and Hu, 2019; Schindler et al., 2020b). The mesocosms were therefore kept water-saturated for 14 days to simulate an intense short-term flood event, allowing anaerobic conditions to form.

Soil respiration and methane emissions

To measure soil CO_2 efflux and CH_4 emissions, a permanent soil collar was installed in each pot. The collars were constructed of 110-mm drainage pipe inserted c. 10 cm into the soil, leaving 2 cm above the soil surface (Floplast Ltd, Kent, UK). All collars were sterilised in 1% bleach solution for four hours, rinsed thoroughly and then installed slightly off centre on the opposite side to the tree in each pot, taking care to minimise soil disturbance and root damage.

Soil CO_2 and CH_4 fluxes were measured in all pots immediately before the flood treatment, using an LGR ultraportable methane analyser (Los Gatos Research, ABB group, San Jose, CA, USA). A customer chamber was constructed to fit onto the existing soil collars in the pots. The chamber consisted of a section of 110-mm drainage pipe (Floplast Ltd, Kent, UK), with a 110-mm double pipe coupler (Floplast Ltd, Kent, UK) at the top and a 110-mm single pipe coupler (Floplast Ltd, Kent, UK) at the bottom end. The top end of the chamber was sealed with a 110-mm socket plug (Floplast Ltd, Kent, UK) while the bottom end was left open to fit over the collar. Gas tubing was fitted into the socket plug to connect the chamber to the instrument. Each measurement lasted 3 minutes with a 30-s dead-band. Soil CO_2 and CH_4 fluxes were measured on days 1, 2, 3, 5, 7, 10 and 14 of the flood treatment. The flooded pots were then removed from flexi-tubs, allowed to drain, and returned to the pre-flood watering regime (see above). Post-flood soil CO_2 and CH_4 fluxes were then measured on days 1, 2, 3, 5, 7, 10 and 14 after the pots were drained.

Nutrient availability

To establish how flooding, N-fertilization and drought history altered plant nutrient availability, specifically N, P, K, Ca, and Mg, I installed two anion and two cation exchange resin strips (PRS probes, Western AG, Canada) in 64 of the pots (eight pots per treatment and species) one day after the end of the flood treatment. The probes were exposed for 14 days, then carefully removed, and gently cleaned of all remaining soil particles using dH_2O and a soft brush. All probes per pot were placed together in a plastic bag and shipped in temperature-controlled packaging to the manufacturer for analysis.

To determine soil total carbon and nitrogen and leaf tissue carbon and nitrogen, a subsample of each dried sample of leaves and soil from the final harvest (see below) were milled to a fine powder using a ball-mill (Mixer Mill MM 200, Verder Scientific UK Ltd, Hope, UK). Milled samples were analysed for total carbon and nitrogen concentration via combustion (Vario EL Cube, Elementar UK Ltd, Stockport, UK) and C:N ratio was calculated from the obtained values.

Final harvest

To quantify above-ground biomass, at the end of the experiments in June 2021, I cut the trees at the soil interface, and a final height measurement of the trees was taken by laying them out on the ground after they were cut. I then separated leaves from branches and stems and dried the leaves and woody biomass at 70°C for seven days before measuring dry weight.

Soil pH was measured directly after harvest. I created a suspension of 10-g of fresh soil and 20-ml of 0.01M $CaCl_2$ in a 50-ml Mason jar and let it stand for 20 minutes (Osman, 2012) before taking a pH reading using a FiveEasy pH/mV benchtop meter (Mettler Toledo, Beaumont Leys, Leicester).

Data analyses

All analyses were performed using R version 4.1.1 (R Core Team, 2021), *RStudio: Integrated Development for R (RStudio Team, 2021)* using the *lme4 (*Bates, Maechler, Bolker, Walker, 2015), MuMIn (Barton, 2020), lmerTest (Kuznetsova, Brockhoff, Christensen, 2017) packages for linear mixed effects models. All results were graphed using the ggplot2 (Wickham 2016) package. Where necessary, data were power- or log-transformed before analysis to meet modelling assumptions.

As the effects of drought were only apparent in the pots with added N (Chapter 4), the present analyses assessed how flooding interacted with the legacy of drought, and the effects of N-addition in a factorial design with two levels for each treatment (flooded vs. not flooded, droughted vs. non-droughted and with N vs. no added N), making eight treatments in total. Separate analyses were conducted for each species. I modelled the effects of flooding, N-addition, and drought history on soil CO_2 and CH_4 fluxes during the two-week flood and subsequent two-week recovery period using linear mixed effects models with factorial N-addition, flood treatment, drought treatment and their interactions as fixed effects, and block and time (days since the start of the flood) as random effects. I then determined the effects of the treatments on soil nutrient ion (N, P, K, Ca, Mg, Zn) exchange rates using linear models. I modelled the exchange rates for each nutrient as a function of factorial N additions, flood treatments, drought treatments, and their interactions.

Relative growth rates for tree stem height and diameter were calculated by dividing the final values for stem height or diameter by the initial values and the number of days in the growth period. The effects of N-addition, flooding, drought history and their interactions on foliar N, foliar biomass, stem height, stem diameter and stem woody biomass were assessed using linear models (*lm* function) for each species separately, fitting N fertilisation, flood, drought history and their interaction as explanatory variables, while block was included as an error term.

All models were simplified by sequential removal of non-significant terms until the most parsimonious model remained. The best fit model was then compared to an appropriate null model (intercept-only) using a likelihood ratio test, and the fit of the model inspected using diagnostic plots (see Appendix C for all tested models). I report the χ^2 and p-values from the likelihood ratio tests for the final model fit and the significance of individual terms using Satterthwaite's approximation to estimate degrees of freedom. All results are reported as significant at $p < 0.05$ and as trends at $p < 0.075$.

4.3 Results

Interactive effects of flooding, N-addition, and drought history on soil gas fluxes

The responses of soil CO_2 and CH_4 fluxes to flooding were shaped by drought history, which differed between species. Soil CO_2 efflux increased markedly with flooding under both species and N-addition tended to enhance the effect of flooding (Appendix C1). However, under poplar, the effect of flooding and N-addition on soil CO_2 efflux was greater in pots with no history of drought (drought x flood x N interaction: $\chi^2 = 9093$; $p < 0.001$; Figure 4.1a), whereas in willow, the effect of flooding and N-addition was greater in pots that had previously experienced drought (drought x flood x N interaction: $\chi^2 = 9173.1$; $p < 0.001$; Figure 4.1b). Interestingly, without flooding, N-addition only enhanced soil CO_2 efflux in willow with no drought history. Thus, in willow, the combined effect of flooding and N-addition was much greater in pots that had experienced drought during the previous growing season (Fig. 4.1b). By contrast, in poplar, the combined effects of flooding and N-addition were much greater in pots that had not experienced drought (Appendix C1).

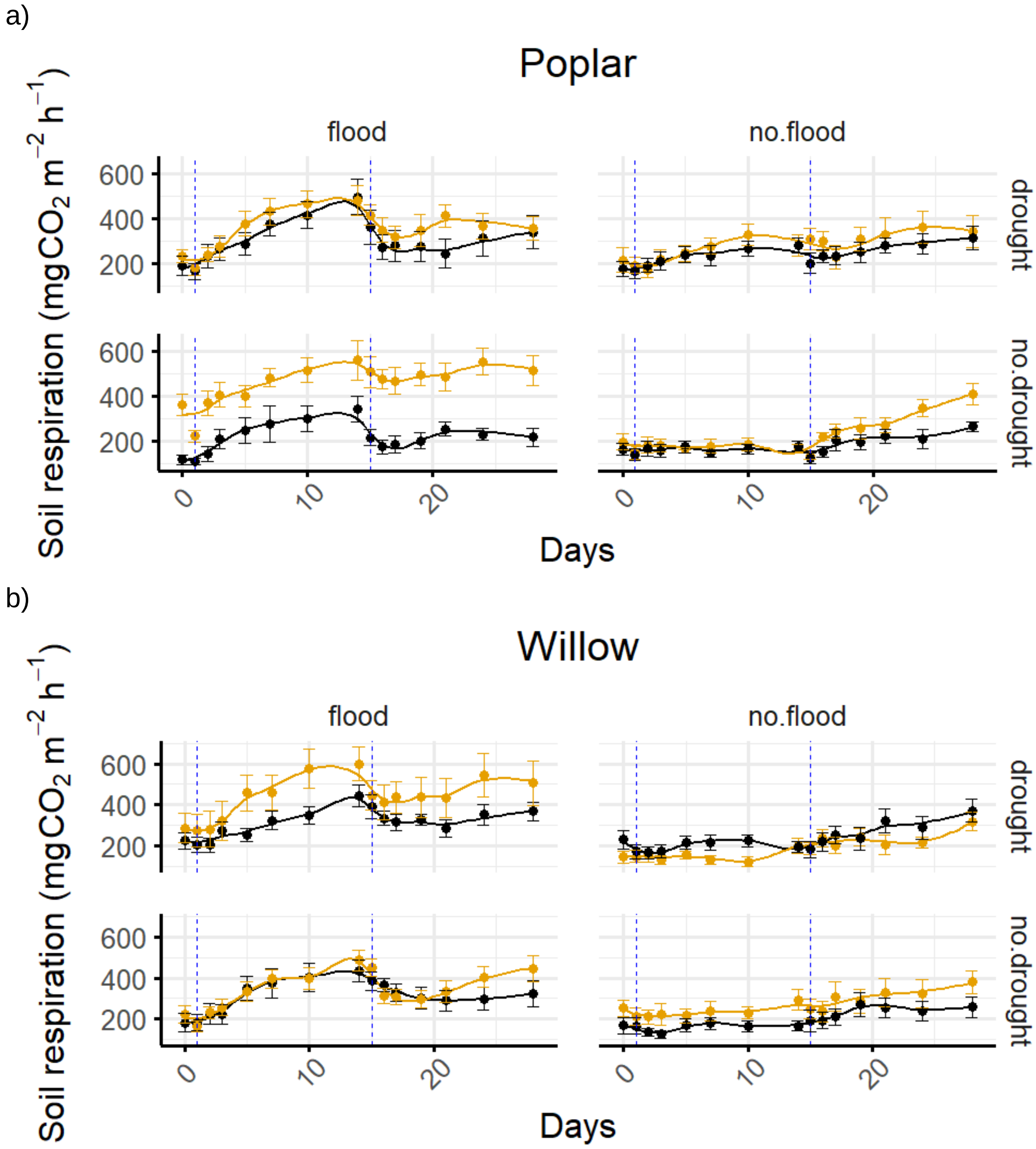


Figure 4.1. *Soil respiration (CO$_2$ efflux) under a) poplar, b) willow during a flooding experiment in mesocosms planted with poplar or willow trees that either had drought history (drought) or had not previously experienced a drought (no.drought). Blue dashed lines show the start and end of the flood treatment, yellow lines and symbols denote trees fertilized with nitrogen (N), black lines and symbols indicate trees without added N. Dots and whiskers represent means and standard errors for n=8, where n is the number of replicates per treatment combination per tree species.*

Soil CH$_4$ fluxes in unflooded soils were low under poplar (treatment means <3 μmol m^{-2} h^{-1}) and largely negative under willow, indicating minor CH$_4$ uptake (Appendix C2), but soil CH$_4$ fluxes increased markedly with flooding under both tree species. Drought history influenced the response of soil CH$_4$ fluxes to flooding and N-addition. In poplar, N-addition enhanced the effect of flooding on soil CH$_4$ fluxes in pots with drought history, whereas in willow, N-addition enhanced the effect of flooding on soil CH$_4$ fluxes in pots that had not previously experienced drought (flood x drought x N interaction; poplar: χ^2

= 529.8; $p < 0.001$; Figure 4.2a; Willow: $\chi^2 = 416.5$; $p < 0.001$; Figure 4.2b; Appendix C2).

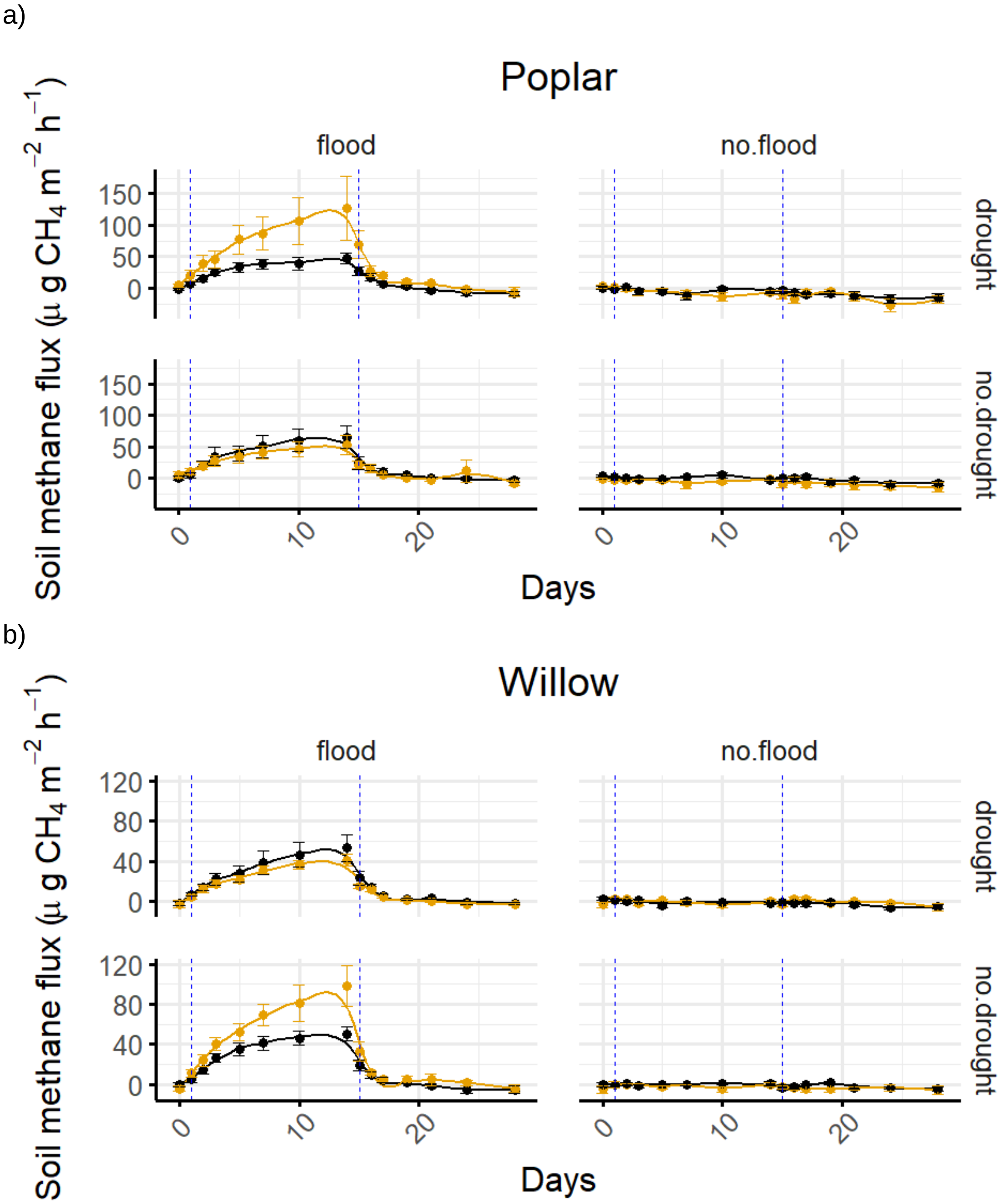


Figure 4.2. *Soil methane (CH_4) fluxes under a) poplar b) willow during a flooding experiment in mesocosms planted with poplar or willow trees that either had drought history (drought) or had not previously experienced a drought (no.drought). Dashed lines show the start and end of the flood treatment, yellow lines and symbols denote trees fertilized with nitrogen (N), black lines and symbols indicate trees without added N. Dots and whiskers represent means and standard errors for n=16, where n is the number of replicates per treatment combination per tree species.*

Interactive effects of flooding, N-addition, and drought history on nutrient availability

At the end of the experiment, soil N and Zn exchange rates were below detection limits in both species. Phosphorus exchange rates were highly variable but were not affected by any treatment. However, the exchange rates of K, Ca, and Mg were influenced by one or more treatments in at least one of the two species (Appendix C3).

Potassium exchange rates under poplar were higher with N-addition but only in flooded pots (N x flood interaction: $F_{3,59} = 5.45$, $p = 0.002$; Figure 4.3a), whereas in willow, K exchange rates were greater with N-addition regardless of flood treatment (N-effect: $F_{1,61} = 31.5$, $p < 0.001$; Figure 4.3a). Calcium exchange rates were unaffected by N addition under poplar and were lower in controls (no drought, no flood) compared to flooded pots and pots with drought history (flood x drought interaction: $F_{3,59} = 5.18$, $p = 0.003$; Figure 4.3b). By contrast, Ca exchange rates under willow were lower in pots with added N compared to pots without N and flooding increased Ca exchange rates in pots without added N (flood x N interaction: $F_{3,59} = 3.82$, $p = 0.014$; Figure 4.3b). Magnesium exchange rates under poplar were unaffected by N-addition but were marginally lower in control pots (no drought, no flood) compared to flooded pots and pots with drought history (flood x drought interaction: $F_{3,59} = 5.11$, $p = 0.003$; Figure 4.3c). Magnesium exchange rates under willow were not affected by any treatment (Appendix C3).

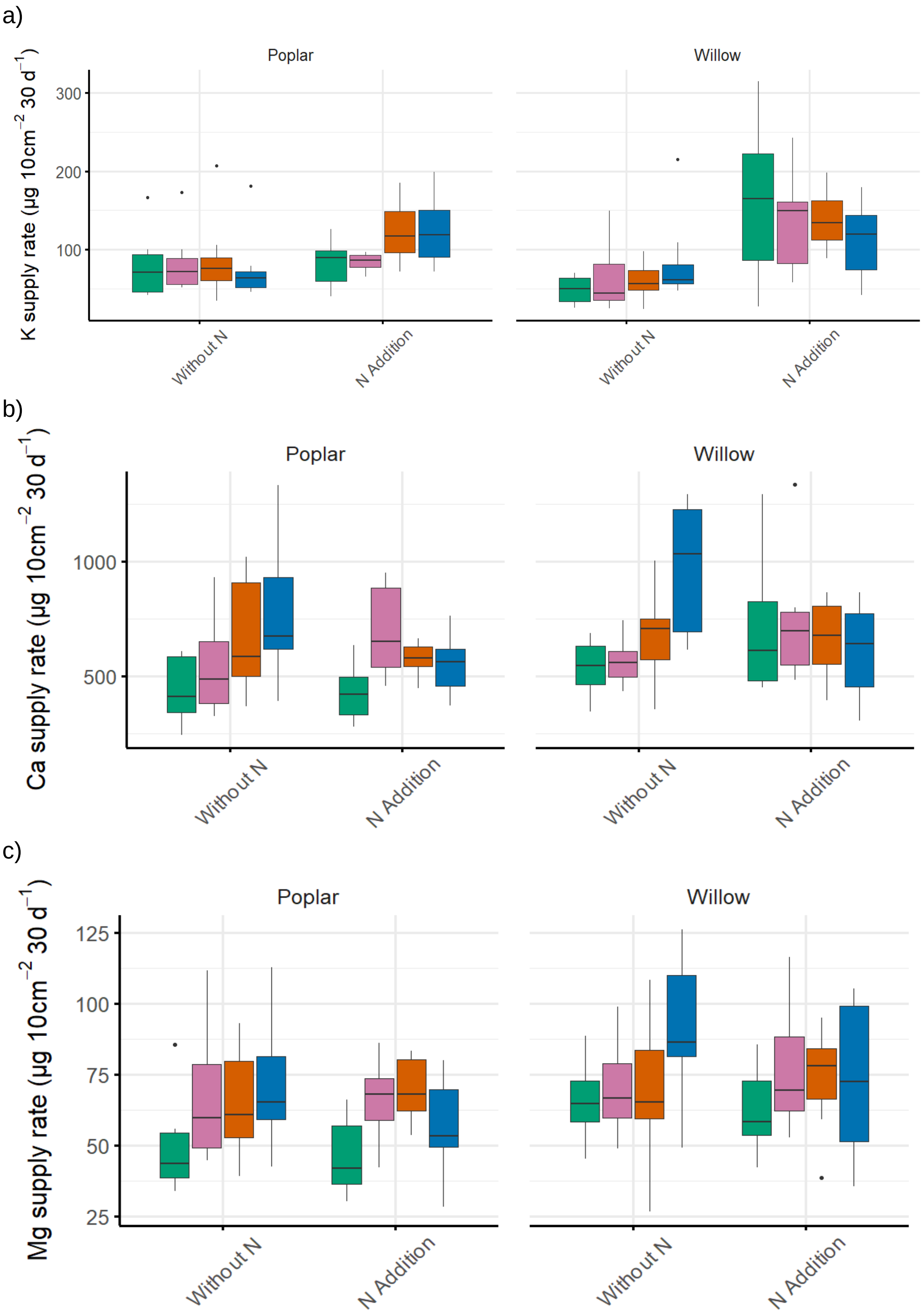
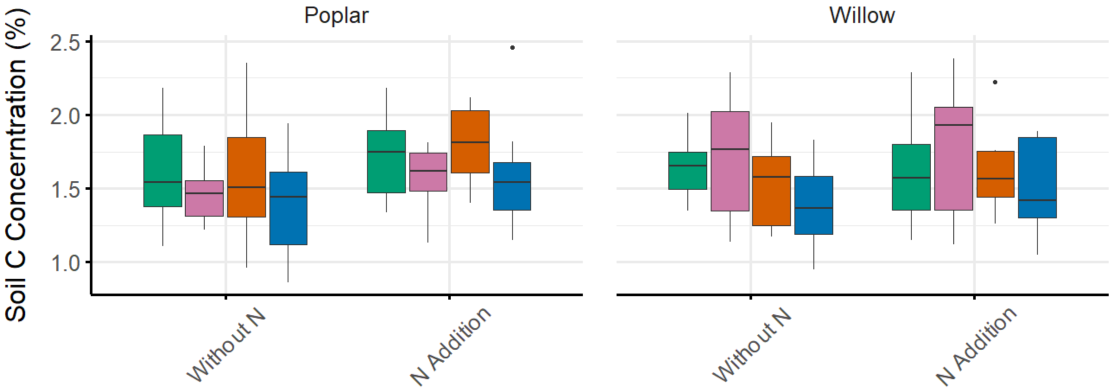


Figure 4.3. *Effects of flood and drought history (green=control, pink=drought, orange = combined drought & flood, blue=flood) on ion exchange rates for* ***a)*** *potassium (K),* ***b)*** *calcium (Ca), and* ***c)*** *magnesium (Mg) under poplar and willow trees in soils amended with nitrogen (N Addition) compared to unfertilised soils (Without N); boxes denote the 25th and 75th percentiles and median lines are given for n = 8, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

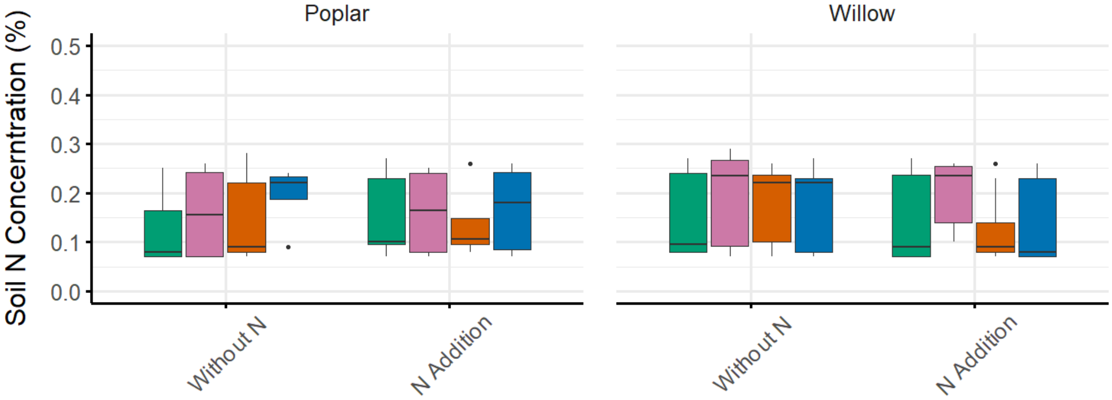
Interactive effects of flooding, N-addition and drought history on soil and foliar nutrients and tree growth

At the end of the experiment after two years of tree growth, there were no apparent effects of any treatment on total soil C and N concentrations or foliar C concentrations (Figure 4.4a, 4.4b, 4.4c; Appendix C4). However, foliar N in poplar was *c.* 57% greater in pots with added N and a history of drought, regardless of flood treatment (N x drought interaction: $F_{7,55} = 14.2$, $p < 0.001$; Figure 4.4a). Foliar N in willow was also *c.* 38% higher with added N in drought treatments, but this increase was only significant in pots that had not been flooded (N x drought interaction: $F_{7,55} = 4.16$, $p < 0.001$; Figure 4.4a). Foliar C:N ratios were also higher with added N in pots with a history of drought (Figure 4.4e & 4.4f; Appendix C4)

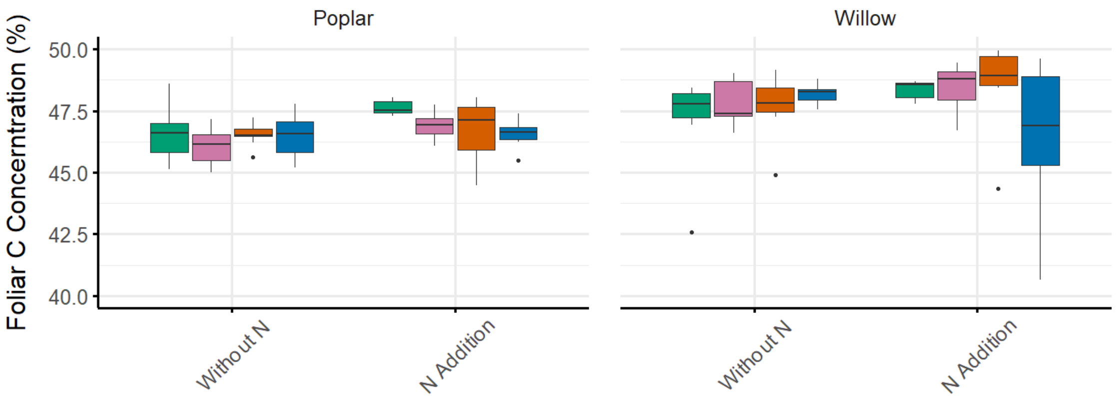
a)



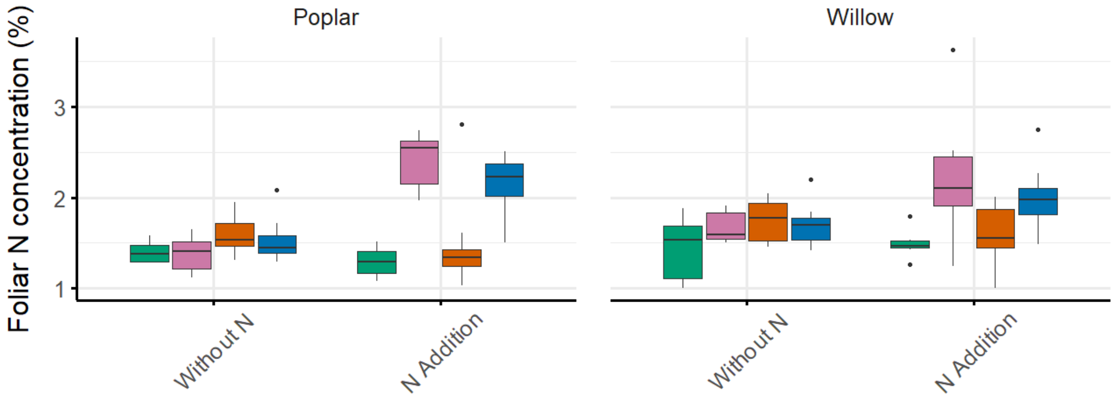
b)



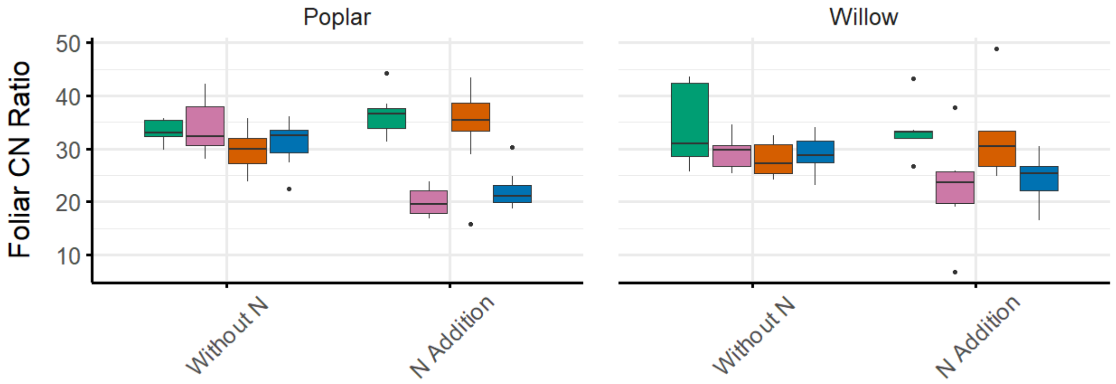
c)



d)



e)



f)

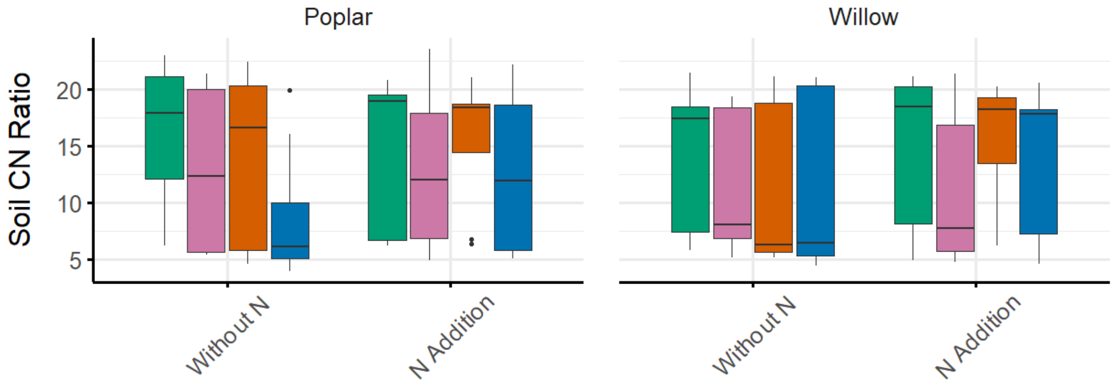


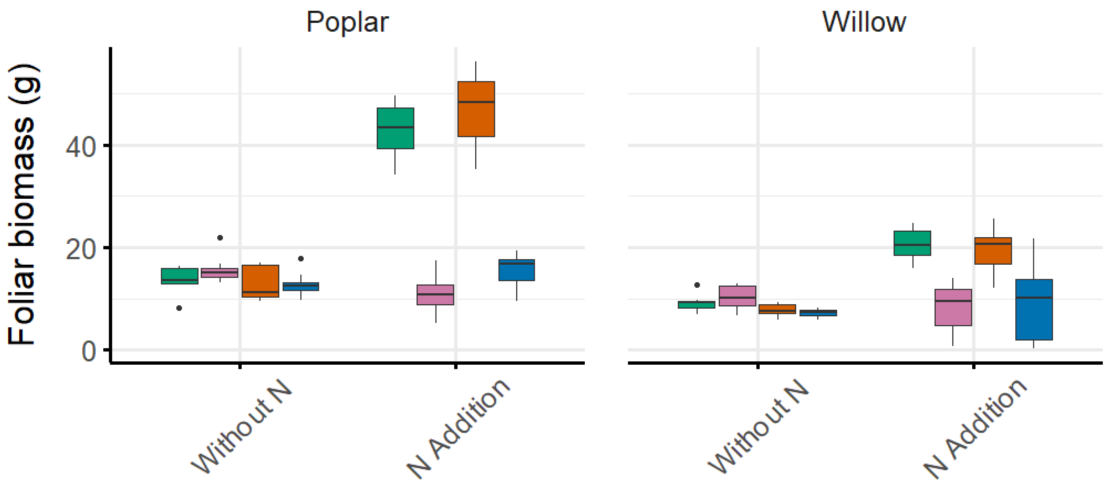
***Figure 4.4**. Effects of flood and drought history (green=control, pink=drought, orange = combined drought & flood, blue=flood) on ion exchange rates for **a)** Soil C Concentration, **b)** Soil N Concentration, **c)** Foliar C*

*concentration, **d)** Foliar N Concentration, **e)** Foliar C:N Ratio and, **f)** Soil C:N Ratio under poplar and willow trees in soils amended with nitrogen (N Addition) compared to unfertilised soils (Without N); boxes denote the 25th and 75th percentiles and median lines are given for n = 8, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.*

Foliar biomass in both species increased substantially with N addition but only in trees that had not experienced drought, and the difference was much more pronounced in poplar (N x drought interaction; poplar: $F_{7,55} = 91.7$, $p < 0.001$; willow: $F_{7,55} = 13.9$, $p < 0.001$; Figure 4.5a; Appendix C5).

The tree height relative growth rate in poplar was unaffected by drought and flooding but was *c.* 56% greater with N addition (N effect: $F_{1,61} = 315$, $p < 0.001$; Figure 4.5b). Similarly, the stem diameter relative growth rate in poplar was unaffected by drought and flooding but increased by *c.* 57% with N addition (N effect: $F_{1,62} = 316$, $p < 0.001$; Figure 4.5c). There was no effect of any treatment on the tree height relative growth rate in willow but stem diameter relative growth rate in willow increased by *c.* 52% with N addition and the increase was smaller in trees that had experienced both flooding and drought (N x flood x drought interaction: $F_{7,56} = 33.3$, $p < 0.001$; Figure 4.4c). Accordingly, stem biomass was greater with added N in both species (N effect; poplar: $F_{1,61} = 23.7$, $p < 0.001$; willow: $F_{1,62} = 719$, $p < 0.001$; Figure 4.4d) but was unaffected by flooding and drought (Appendix C5).

a)



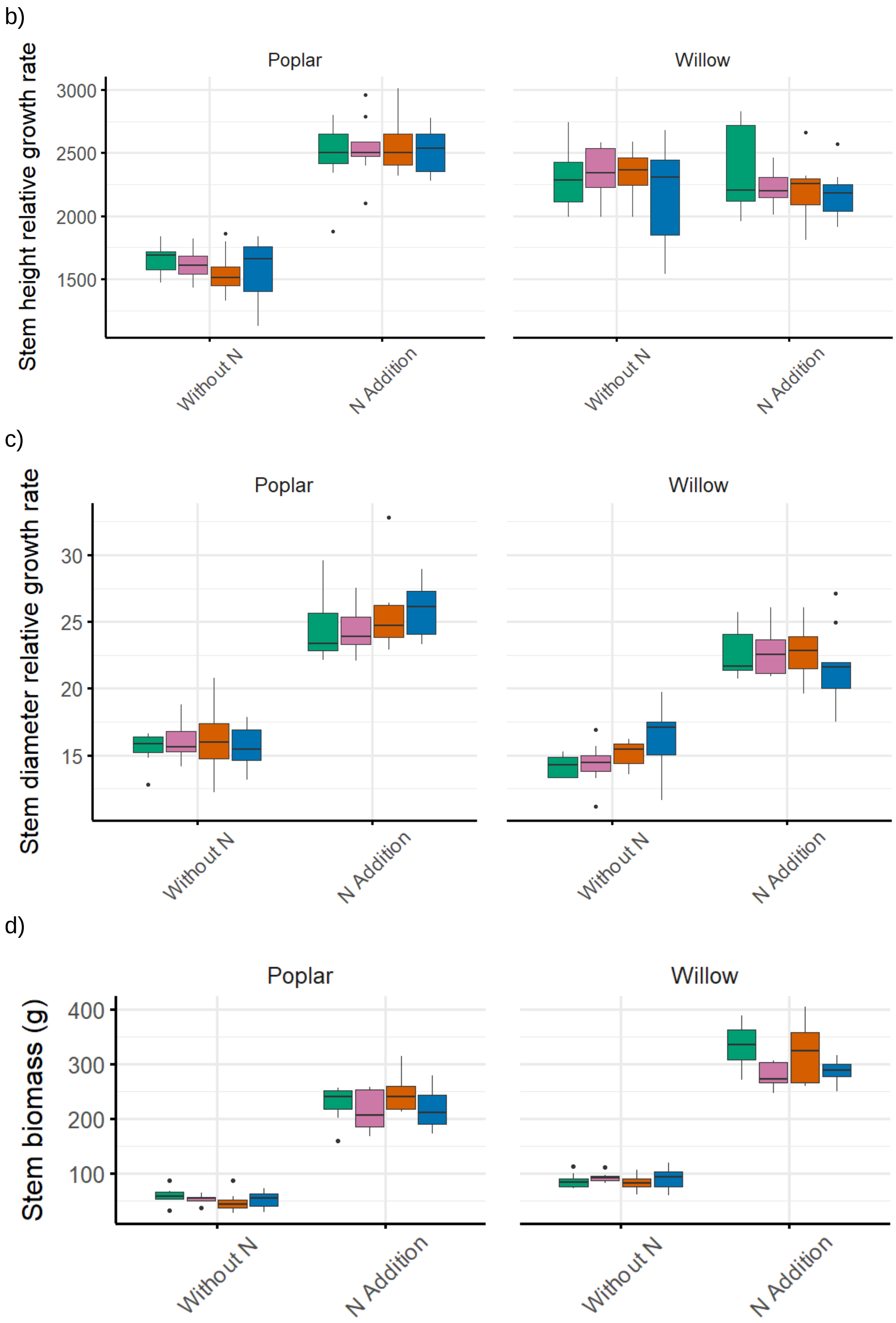


Figure 4.5. *Figure 4.5 The effects of flood and drought history on* ***a)*** *foliar biomass,* ***b)*** *stem height relative growth, c) stem diameter relative growth, and d) stem biomass of poplar and willow trees (green=control, pink=drought, orange = combined drought & flood, blue=flood) in soils amended with nitrogen (N Addition) compared to unfertilised soils (Without N); boxes denote the 25th and 75th percentiles and median lines are*

given for n = 8, where n is the number of replicates per treatment combination per tree species, whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.

4.4 Discussion

My study demonstrated that interactions between flooding, N-fertilization and historic drought can influence soil CO_2 and CH_4 fluxes under SRC crop species. Importantly, drought history affected GHG fluxes during flooding, with effects differing strongly between species. In addition, fertilization with N increased the impact of flooding on both CO_2 and CH_4 fluxes. However, N-fertilization also ensured substantial increases in growth and biomass of both species, regardless of whether they were subjected to flooding or drought.

Numerous studies have assessed how N-addition or water availability individually influence GHG emissions from SRC crops. However, to my knowledge, my work is the first to determine how fertilization and flooding interact to influence soil GHG fluxes under SRC crops. Furthermore, I evaluated for the first time how drought history influences the effect of flooding and N-addition on greenhouse gas fluxes under SRC crops and showed remarkable differences between species. Here I interpret my findings within the context of bioenergy crop management and discuss their wider implications for CO_2 and CH_4 dynamics in SRC crops.

Interactive effects of flooding, N-addition, and drought history on soil gas fluxes

In support of my first hypothesis, flooding significantly increased both CO_2 and CH_4 emissions from the soil and N-addition enhanced the effect of flooding. The increase in CO_2 emissions with flooding was consistent for both species, and N-addition enhanced the effect of flooding (Figure 4.1a; Figure 4.1b). Often soil respiration decreases with flood events as soils become waterlogged, leading to anoxic conditions, thus limiting oxygen availability for much of the microbial community and root functions (Han et al., 2018). However, the extent to which CO_2 emissions increase during flood events likely depends upon carbon mineralisation by the microbial community (Guntiñas et al., 2009), whereby emissions often increase with the duration and intensity of the flood event (Guntiñas et al., 2009; Khalid, Shaaban and Hu, 2019) Frequently flooded soils have lower total organic C concentrations (Saint-Laurent, Arsenault-Boucher and Berthelot, 2019), which might result from increased mobilisation of organic carbon in soils exposed to more intense flooding (Majidzadeh et al., 2017), as a shift from aerobic to anaerobic conditions caused by intensive flood increases dissolved organic C (DOC) in soils (Sao, Praise and Watanabe, 2023). Repeated flooding allows for continual breakdown of soil aggregates leading to mobilisation of previously inaccessible carbon pools in turn increasing DOC which is lots through microbial mineralisation and CO_2 fluxes (Wang et al., 2021a), Interestingly, land use has been shown to play a significant role in CO_2 emissions during flooding, as agricultural land and grassland have higher emissions than woodland, which has been attributed to fertilisation of agricultural land (Guntiñas et al., 2009). My findings provide direct evidence that N-addition boosts CO_2 emissions from the soil during flood events in SRC crop plants (Figure 4.1a; Figure 4.1b). Given that SRC crops are often grown on marginal land prone to flooding (Caslin, Finnan and McCracken, 2011) and require fertilization after establishment (DEFRA, 2004), the effects of N-addition on CO_2 emissions need to be considered when calculating the carbon balance of SRC crops.

The importance of water table level in CH_4 emissions is well-established, where a water-table above 10-20 cm depth results in a substantially higher production of CH_4 (Zona et al., 2013). The lack of oxygen in flooded soils creates conditions favourable to anaerobic decomposition of organic matter leading to release of CH_4 from methanogenic organisms (Topp and Pattey, 1997). Increased CH_4 emissions have been observed in various studies including grasslands (Sánchez-Rodríguez et al., 2019), riparian forests (Schindler et al., 2020a), and rice crops (Bronson et al., 1997; Schimel, 2000; Yao et al., 2012). By contrast, CO_2 emissions under flood conditions are less commonly studied but increased soil CO_2 efflux has been reported in grassland ecosystems (Sánchez-Rodríguez et al., 2019). However, the extent of increases in GHG emissions can vary greatly depending on the form of N-addition, and some studies have also observed no effect or even a decrease in emissions in response to combined flooding and N-fertilization (Minami, 1995; Cai, Shan and Xu, 2007; Bhattacharyya et al., 2012; Tang et al., 2018; Wang et al., 2018). In my study, the increase in GHG fluxes with N-addition was influenced by both drought history and species identity, indicating that the effects of extreme weather on the GHG balance of SRC crops could depend greatly on the choice of species (Figure 4.2a; Figure 4.2b). Higher CO_2 emissions with flooding and N-addition could be a result of increased population size of soil organisms involved in carbon turnover under greater N availability, which is commonly seen during drying-rewetting cycles (Li et al., 2018). Furthermore, N-addition increases plant biomass, root respiration and thus organic matter inputs, providing more substrate for soil microorganisms. Previous work has demonstrated that N-addition during a flood event indirectly intensifies CH_4 emissions from soils by enhancing plant growth and substrate availability to methanogens (Schimel, 2000). Methanogenic organisms have a high N requirement, and react to N-addition by increasing population density and activity, which in turn increases CH_4 emissions (Yao et al., 2012). Indeed, N-addition alone can weaken the CH_4 sink strength of unflooded soils by reducing CH_4 uptake, a phenomenon referred to as the N inhibition effect (Mosier et al., 1991). Hence, in my study flooding interacted with N fertilization to boost soil GHG emissions under SRC crop plants.

It is possible that differences in root biomass, which were not measured in this study, may have contributed to some of the variation in CO_2 and CH_4 fluxes. Differences in root biomass between treatments and species would directly affect CO_2 emissions through differences in root respiration rates. In addition, greater root biomass can increase the rate at which methanogenesis occurs by increasing oxygen consumption, leading to a faster depletion of oxygen (Wang et al., 2021a). As strictly anaerobic conditions are required for methanogenic production of CH_4, this depletion of oxygen in the soil by roots could boost CH_4 emissions.

Interestingly the findings of this study did not support my second hypothesis, as drought history did not reduce soil CO_2 or CH_4 emissions. Instead, drought history and flooding interacted to influence soil GHG emissions, but the effects differed strongly between species. In poplar, drought history dramatically increased CH_4 emissions in flooded soils (Figure 4.2a) but had little effect on CO_2 efflux (Figure 4.1a). By contrast, drought history in willow increased soil CO_2 efflux in flooded soils (Figure 4.1b) but did not affect CH_4 emissions (Figure 4.2b). Overall, drought history had the greatest effects on GHG fluxes under flooding in N-fertilized pots (Figure 4.1a; Figures 4.1a; Figure 4.2a; Figure 4.2b), which is explained by the greater severity of drought in N-fertilized pots due to higher water requirement of the larger trees (Chapter 3). The interactions between drought and N-fertilization are therefore not considered further here. However, the distinct patterns in soil GHG fluxes under these two common SRC species are intriguing, and, to my knowledge, no studies have previously revealed this phenomenon under SRC crops. In poplar, the higher CH_4 emissions observed in flooded soils with a history of drought (Figure 4.2a) could be a result of increased N content and reduced N mineralisation in soils during drought (Deng et al., 2021), which would provide additional N to methanogenic organisms post-drought, thus increasing CH_4 fluxes during the subsequent flood (Topp and Pattey, 1997). However, I found no evidence of increased soil N availability after the drought (Chapter 3). Instead, decomposition of plant material that senesced during the drought could have provided additional substrate to methanogens. It is possible that drought history also affected soil CO_2 emissions during flooding under willow (Figure 4.1b): higher CO_2 emissions in flooded soils with a history of drought occurs because the drought period leads to the breakdown of soil aggregates, allowing microbial communities access to carbon that was previously unavailable (Liu et al., 2022). However, the influence of drought history on GHG emissions during flooding needs further investigation to elucidate the mechanisms underpinning the contrasting results between species.

Flooding and drought history influence nutrient availability in SRC crops

Strong changes in soil moisture, such as during droughts and floods, can alter nutrient availability even after equilibrium has returned (Zhang, Wang and Li, 2022). The findings of my study partially support my third hypothesis showing that both flooding and drought history influenced the availability of K, Ca, and Mg. However, only the availability of Mg declined after the flood (Figure 4.3c), whereas K (Figure 4.3a) and Ca (Figure 4.3b) availability generally increased, depending on tree species and N-addition.

Although the increased availability of K with N-addition is a direct result of K added in the N-addition fertilisation treatment, flooding in poplar further increased K availability (Figure 4.3a). To my knowledge, my study is the first to assess the effects of flooding on nutrient availability in soils under SRC crops. However, work in rice paddies has demonstrated increases in exchangeable K in flooded soils (Jahan et al., 2013) as K is highly mobile in wet soils (Schneider, 1997). Poplar appears to take up K at a relatively slow rate (Tüfekçioğlu et al., 2005), which would explain greater K availability under poplar but not under willow. The increased exchangeable Ca in flooded soils with drought history in poplar (Figure 4.3b) might be explained by greater CO_2 concentrations in pore water, creating carbonic acid which would release Ca held in other forms (Curtis, Monger et al., 2015). Similarly, Ca release by carbonic acid in flooded soils with N-addition under willow could counteract the effects of soil acidification by N fertilizers, which binds Ca to the soil surface (Tian and Niu, 2015). Finally, although Mg exchange rates were not significantly affected by any treatment, they were marginally lower in pots exposed to both drought and flood compared with controls (Figure 4.3c), which is consistent with earlier studies showing that soil moisture content plays an important role in the movement of Mg in soils, whereby both drought and flood can reduce Mg availability (Dunham and Nye, 1976). Thus, compound extreme events are likely to alter the availability of multiple nutrients for SRC crops, but predicting the effects of combined flood and drought events is difficult due to the various potential mechanisms and differences between species.

Flooding, N-addition and drought history affect foliar nutrients and tree growth

My findings partially support my final hypothesis, as N-addition increased N concentration in leaves (Figure 4.4c), leaf biomass (Figure 4.5a) and woody stem biomass (Figure 4.5d) in both species regardless of drought or flood treatments. However, contrary to my predictions, flooding did not influence tree growth (Figure 4.5b; Figure 4.5c) or biomass (Figure 4.5d), and drought only reduced foliar biomass (Figure 4.5a), but not stem diameter (Figure 4.5c) or height growth (Figure 4.5b).

Although SRC crops are considered low-input, where little nutrient addition is required (The Scottish Agricultural College, 2008), my findings add to previous studies highlighting significantly increased yields with N-fertilization (Aronsson, Rosenqvist and Dimitriou, 2014b). As most SRC crops are high-yielding, especially willow and poplar, they have a high N requirement to facilitate continued fast growth (Djomo, Kasmioui and Ceulemans, 2011). A major benefit of fast woody biomass growth is carbon sequestration (Rytter, Rytter and Högbom, 2015), and thus, increasing N-addition to meet growth needs allows plants to allocate more carbon resources to both woody biomass and leafy biomass which enhances carbon sequestration (Janssens et al., 2010). My results show that N-fertilization alone doubled the biomass of both species during the first two years of growth, which could translate to substantial carbon benefits in field-grown crops on marginal land.

Notably, neither flood nor drought history independently affected foliar N concentrations (Figure 4.4d) or growth (Figure 4.5b; Figure 4.5c) in either species. However, drought in the previous growing season reduced foliar biomass (Figure 4.5a), and the interaction between N-addition and drought history substantially increased N concentration in leaves (Figure 4.4b) in both species. Higher foliar N concentrations but reduced foliar biomass (Figure 4.5a) in trees with a history of drought suggest that the trees have taken up the same amount of N as previously once the regular watering regime was reinstated, leading to an increase in N concentrations in foliage compared with plants not exposed to drought. The reverse of this effect (lower foliar N concentrations in trees with increased leaf biomass) is often referred to as the dilution effect (Gonzalez-dugo, Durand and Gastal, 2010). Interestingly, some studies have suggested that N-addition facilitates a faster recovery from drought conditions via increased photosynthesis, which requires a higher N concentration in leaves (Saud et al., 2017; Zhong et al., 2019). It is nonetheless noteworthy that N-fertilized trees that had experienced a drought in the previous growing season had lower foliar biomass the following spring. This finding suggests that even a short drought could have longer-term effects on SRC growth by reducing leaf biomass in the subsequent growing season.

Although there were no significant responses to water availability in height or diameter growth in either species, stem diameter in N-fertilized willow was smaller in trees subjected to drought and flooding, further supporting my hypothesis that compound extreme events, represented here by a 28-day drought followed by a 14-day flood seven months later, will negatively impact yield. It is important to note that the full effects of compound extreme weather events on growth may not have been captured by this study. The definition of an extreme weather event is subjective where duration and intensity vary depending on location; however, it can be generally defined as a rare event in a specific location at a certain time of year (IPCC, 2021). In addition, both tree species are temperate deciduous trees with a winter dormancy period and trees were harvested very early in the final growing season. It was therefore not possible to establish if woody biomass and tree growth would have been significantly impacted by flooding at a later stage in the growing season. Nonetheless, my findings add to a body of literature highlighting that even short drought exposure can have a significant effect on biomass indicators (Macalpine, 2019) and demonstrate for the first time that compound events can substantially alter growth and GHG emissions in SRC crops.

4.5 Conclusions

To my knowledge this study is the first to assess the interactive effects of flooding, drought, and N-addition on soil GHG fluxes and nutrient availability under SRC crop species. Understanding how predicted extremes in water availability interact with N-addition might influence plant-soil GHG dynamics and crop yield is essential to determine whether SRC crops can deliver a carbon-neutral energy supply. My findings demonstrate that soils under poplar and willow showed opposite changes in GHG fluxes in response to the combined influences of flooding and historic drought, which were amplified by N-fertilization. In particular, drought history determined the magnitude of CO_2 or CH_4 fluxes during a flood event, which suggests that the legacy effects of one extreme weather event could influence the response of the system to the next event. Importantly, although N-addition could compensate for yield declines due to flooding, it also greatly enhanced CO_2 and CH_4 emissions from soils, which could reduce the carbon benefits of SRC crops. Finally, the interaction between treatments also highlighted possible pathways for nutrient leaching. Overall, my work exposes the need to fully understand how land management will influence both growth and GHG emissions under a changing climate. As many soils need N-fertilization and extreme weather events are expected to become more frequent, future studies should focus on SRC species selection to mitigate soil GHG emissions and maximise carbon sequestration.

5. Thesis Discussion

5.1 Introduction

Although the carbon neutral status of short rotation coppice (SRC) tree species such as willow and poplar is well documented (Caslin, Finnan and McCracken, 2010; Lockwell, Guidi and Labrecque, 2012; Verlinden et al., 2013; Pacaldo, Volk and Briggs, 2014; Ferré and Comolli, 2018; Ventura et al., 2019), studies often focus on one key influencing variable, e.g. mycorrhizal fungal colonisation (Hrynkiewicz et al., 2012; Fang et al., 2020a), nutrient additions (Aronsson, Rosenqvist and Dimitriou, 2014b; 2014a; Ugilt et al., 2018), or the increase of extreme weather events such as droughts (Doffo et al., 2017) or floods (Zimmer et al., 2012). However, most studies have not considered how interactions among several aspects of climate change or land management will alter crop yield or soil greenhouse gas (GHG) emissions under SRC bioenergy crops. As a result, there remain considerable uncertainties around the carbon budget and GHG dynamics of SRC crops. Creating integrated systems to provide carbon neutral bioenergy sources that do not enhance soil GHG emissions under predicted climate change scenarios is a pressing need (HM Government, 2011). A clearer understanding is needed of how interactions between the soil fungal community, nutrient additions and soil moisture levels allows the opportunity to expand the area dedicated to these crops, without creating competition with food production (Lockwell, Guidi and Labrecque, 2012).

The aim of this thesis was to establish how GHG emissions and carbon storage of temperate SRC crops might be impacted by associations with mycorrhizal fungi, fertilization, and strong changes in soil water availability (drought and flood). To achieve this, I measured soil respiration under two UK native crop species (willow and poplar) inoculated with arbuscular mycorrhizal fungi (AMF) fertilized with nitrogen, phosphorus or both nutrients. I first quantified how each nutrient treatment, AMF treatment and their interactions influenced soil CO_2 fluxes and tree growth during crop establishment (Chapter 2). I then expanded upon this knowledge base by establishing how soil respiration under the two species was impacted by a strong drought during the second growing season (Chapter 3). Finally, by simulating a short but intensive flood event at the start of the third growing season, I aimed to establish how flooding affected soil CO_2 and CH_4 fluxes, how nitrogen fertilization altered GHG emissions, and whether drought history modified the effects of flooding (Chapter 4). I also harvested all trees to establish which treatments affected crop biomass. I hypothesised that interactions between N-addition, drought and flood would have significant influence of growth and carbon dynamics of SRC, specifically CO_2 and CH_4. The work presented in this thesis offers convincing evidence that carbon dynamics were heavily influenced by interactions between N-fertilization and extreme climate events, far more so than any treatment in isolation.

5.2 Key findings

Soil GHG fluxes

Quantifying soil CO_2 and CH_4 fluxes under SRC crop species was a key aim of my studies as it contributed to the main research objective of the thesis: to establish the interactive effects of fertilization, AMF inoculation and extreme weather events on carbon dynamics in SRC crops. My chosen tree species were the most commonly grown lignocellulose species grown as SRC in temperate climates (Oliver, Finch and Taylor, 2009) and my results clearly show they are high-yield, low-input species that become a carbon store before the first coppice (Amichev et al., 2012). Nonetheless, soil moisture content (Tripathi et al., 2018) and nutrient availability (Ugilt et al., 2018) have a sizeable impact on the carbon dynamics in these SRC crops, and my studies demonstrate that crop fertilization and large changes in soil water content interact to influence soil carbon dynamics and tree biomass (Chapters 2,3, 4). In addition, I demonstrated that soil CO_2 emissions increased substantially with both AMF inoculation and N-addition (Chapter 2), which is reflective of the increased tree biomass (Moscatelli et al., 2008; Lagomarsino et al., 2013; Rewald, Kunze and Godbold, 2016; Berhongaray et al., 2017; Ventura et al., 2019). The increase in soil respiration with AMF inoculation has been well established in studies of other plant species, where a greater allocation of plant carbon (C) to AMF allows the formation of large hyphal networks (Lang et al., 2020) combined with a rapid turnover of fungal mycelium (Lin et al., 2017) creating a high turnover of soil C (Driver, Holben and Rillig, 2005). However, I demonstrated that in the absence of nutrient addition, AMF inoculation increased respiration in poplar trees but not in willow, highlighting a clear difference between species in the influence of AMF on soil carbon dynamics (Chapter 2).

Although both tree species are known to form relationships with AMF, my findings contrast with previous studies reporting that the effects of AMF inoculation are greater in willow than in poplar (Fillion et al., 2011). AMF are often generalist species that can colonise many plant species, whereas plants have more specialised preferences (Smith and Read, 2008d). Different willow hybrids have distinct abilities to form associations with AMF (Sumorok and Kiedrzynska, 2007; Rooney et al., 2009; Fillion et al., 2011; Hassan et al., 2014) and the choice of willow variety in my study could have influenced AMF colonisation success. Nonetheless, it is important to note that AMF inoculation also enhanced biomass growth in unfertilized trees but reduced biomass growth in N-fertilized trees during the first growing season (Chapter 2). Continuous measurements of soil GHG fluxes in the field are required to establish whether the enhanced tree biomass offsets the greater release of soil C through respiration. My results suggest that interactions between AMF and fertilization regimes could influence the carbon balance of SRC crops. Hence, the ability of different species and cultivars to associate with AMF could be an important consideration for land managers deciding on fertilization regimes during SRC crop establishment.

Comparing the carbon balance of SRC crops inoculated with AMF or fertilized with N could be valuable to inform land management decisions but this necessitates future field trials, rather than the pot studies I conducted for my research. Growing the trees in pots was necessary to create controlled conditions for my experiments but would have influenced carbon budgets and the strength of some treatment effects. First, as the trees were grown in individual pots, the soil volume available for root extension and AMF nutrient foraging was severely restricted, which likely diminished the positive effects of AMF inoculation on tree growth and biomass. Second, as the trees were grown in poly-tunnels air temperatures during the day were often higher than ambient, particularly during the growing season (spring and summer). Although soil respiration measurements were conducted at a similar time within each experimental block (to account for temporal variation during the measurement phase) and carried out in the morning before the temperature in the polytunnels rose substantially above ambient, the higher air temperatures were nonetheless likely to influence tree growth and microbial activity (Weih, 2001; Hanson et al., 2005; Hawkes et al., 2008; Chen et al., 2013; Soares et al., 2019). Finally, soil respiration rates during the first few months of the study may have excluded a proportion of root respiration because the soil collars used for measurements of soil CO_2 efflux were placed deeper than in previous studies. Soil collars are commonly installed to a depth of c. 2 cm as soil respiration can decrease with increased depth of the collars (Wang et al., 2005; Li et al., 2019), whereas I installed them to a depth of 10-cm, which may have contributed to the minor effect of AMF on respiration rates during the first growing season. The reason was because I installed the collars when repotting the cuttings into their final 18-L pots, when all roots and the inoculated soil mix were deeper than 10-cm, meaning the collars needed to be inserted to this depth to detect treatment differences. However, the influence of soil collar depth would have been consistent across all treatments, and roots were abundant throughout the pots by the end of the growing season. Thus, the influence of AMF inoculation on soil carbon dynamics, tree growth and biomass gain in the field could be much greater than the effects I measured in my pot experiments, which warrants future investigation using field-based measurements.

My drought experiment in Chapter 3 demonstrated that fertilization with N increased soil CO_2 release upon rewetting in both species. This finding is in contrast with previous studies reporting lower soil respiration rates with N-addition in forests (Janssens et al., 2010), and highlights the need for further investigation of the after-effects of drought on soil carbon dynamics under SRC crops. Although the effects of N-addition on soil respiration were short-lived, my experiment demonstrates that soil carbon dynamics are more sensitive to intense drying-rewetting events under N-addition compared with unfertilized conditions (Erinle, Bengtson and Marschner, 2021). The more intense effects of the drought in N-fertilized trees can be attributed to greater tree biomass and therefore water-use (Chapter 3). Measurements of plant water potential and an extended drought period in unfertilized trees might have allowed me to measure rewetting responses to similar drought intensity across treatments. Although it was not possible to adapt the experiment in this way due to delays caused by Covid-19 lockdown restrictions, my findings suggest that drought events will have greater impacts on soil carbon dynamics under fertilized SRC crops, which could have important implications for the whole-crop carbon balance under the projected increase in hot dry summers in the UK (Met Office, 2021). During the month after rewetting, the interaction between N-addition and AMF inoculation increased soil CO_2 emissions in both species, but N-addition reduced the stimulatory effect of AMF on soil respiration in willow (Chapter 3). As there was no influence of the individual treatments, these increases in soil respiration cannot be explained by the greater tree biomass in fertilized or inoculated trees. Several studies highlight how AMF can mitigate impacts of drought on soil respiration, which is thought to be largely due to improvements in soil structure, conferring greater resilience to changing soil moisture content (Wright and Nichols, 2002). My findings add to this by demonstrating that AMF inoculation could improve the resilience of belowground processes to drying-rewetting cycles. The reduction in the stimulatory effect of AMF with N-addition in willow could be linked to a reduction in the requirement for AMF to expand their hyphal networks to forage for nutrients (Camenzind et al., 2016). My findings therefore highlight a new line of enquiry to assess whether N-fertilization could retard the recovery of soil processes after drought by reducing AMF hyphal abundance.

Finally, my flood experiment in Chapter 4 demonstrates that flooding of SRC crops boosts GHG emissions from the soil. Although it is well-established that CH_4 emissions increase with the duration of flooding (Schimel, 2000; Yao et al., 2012; Deng et al., 2021), I found that soil CO_2 emissions also increased during the flood, and N-addition enhanced the effect of flooding on soil GHG emissions in both species. This unusual finding is likely explained by greater microbial C mineralisation with nutrient additions, which has been observed in other agricultural systems (Guntiñas et al., 2009). It is also possible that AMF inoculation played an important role in maintaining soil functioning during flooding (Li et al., 2011; Wang et al., 2016; Diagne et al., 2020). Unfortunately, as the AMF and N-addition treatments in my study were confounded, it was not possible to separate the potential effects of AMF vs. N-addition and new experiments are needed to establish whether AMF influence soil GHG emissions in flooded SRC crops. Nonetheless, although N-addition mitigated reductions in crop yield because of extreme weather events, increased GHG fluxes were also strongly associated with N fertilization.

The most intriguing finding of my flood experiment is the impact of drought history on soil CO_2 and CH_4 emissions (Chapter 4). Drought legacy effects were observed in both species, despite the trees appearing to have fully recovered from the drought during the previous growing season. The legacy effect of drought on soil GHG emissions was not only strongly species-dependent, but opposite patterns for CO_2 and CH_4 release during flooding were observed between species: In willow, CO_2 emissions were higher and CH_4 emissions were lower compared to trees with no drought history, whereas in poplar, CH_4 emissions were higher and CO_2 emissions were lower. These results are surprising and remain unexplained. However, it is clear that in both cases, drought history has the potential to modify GHG emissions during flooding, and future research should focus on identifying the underlying mechanisms.

Nutrient availability

Understanding how nutrient amendments and soil moisture influenced nutrient exchange in soils under SRC crop species was a valuable area of study in my thesis, as a greater understanding of changes in plant available nutrients after extreme weather events provides insights into crop and soil health under future climate change. The ion exchange resins (PRS probes) I employed provide a better measure of plant-available nutrients than standard soil extractions, and allowed me to assess the exchange rates of multiple nutrients simultaneously (Hangs et al., 2002). The use of PRS probes has become more common in soil studies focussing on nutrient additions and soil moisture (Miller, Bremer and Curtis, 2016), and my results clearly showed the importance of interactions between fertilization, AMF inoculation and extreme weather events for nutrient availability. The lower nutrient exchange rates in soils under fertilized trees are unsurprising, as they indicate greater uptake of nutrients to support enhanced biomass growth with N-addition (Bänziger, Edmeades and Lafitte, 1999; Laghari et al., 2016; Fabio et al., 2018; Nordborg et al., 2018; Ugilt et al., 2018). However, the differences in nutrient availability under droughted, flooded or AMF inoculated trees provide insights into the nutrient requirements of SRC crops under different conditions.

In my drought experiment (Chapter 3), the lower exchange rates of calcium in soils inoculated with AMF indicate improved plant uptake of a vital nutrient for improving water use (Liu et al., 2022), which could help alleviate the impacts of drought in SRC crops. Interestingly, my flood experiment (Chapter 4) then showed that flooding increased calcium exchange rates, which may be due to interactions between water and higher soil CO_2 levels, which create carbonic acid and release Ca into the soil (Curtis, Monger et al., 2015). Overall my findings add to previous studies highlighting that large changes in soil moisture can alter nutrient availability and have legacy effects even after the return to normal moisture levels (Zhang, Wang and Li, 2022; Tripathi et al., 2018).

Tree biomass

Understanding how nutrient additions, AMF inoculation, and extreme weather events interact to affect tree growth and biomass is important to inform SRC establishment and management, especially on marginal land (Ens, Farrell and Bélanger, 2013; Basche and Edelson, 2017; Kern et al., 2018). Woody biomass is of particular interest in SRC tree species as it has high energy and carbon content (Oliver, Finch and Taylor, 2009), which are essential for an economically viable and carbon neutral bioenergy crop (Ferré and Comolli, 2018). An important aim of this thesis was to establish if maintaining crop yield via nutrient addition to combat less favourable abiotic conditions altered carbon dynamics under SRC crop species. The growth (stem height and diameter) and biomass measurements I made demonstrated that N-addition increased all measured biometrics (Aronsson, Rosenqvist and Dimitriou, 2014b; Nord-Larsen, Sevel and Raulund-Rasmussen, 2015; Dimitriou and Mola-Yudego, 2017; Hoeber et al., 2017; Ventura et al., 2019), which in turn increases overall carbon storage (Verlinden et al., 2013; Rytter, Rytter and Högbom, 2015; Ventura et al., 2019). However, I also demonstrated that AMF-inoculation enhanced stem diameter growth and woody biomass in unfertilized trees of both species during the first growing season (Chapter 3), indicating that AMF inoculation could reduce the need for fertilizer use to boost yield and aboveground carbon storage in SRC crops grown on nutrient poor soils. Given my promising initial results showing biomass increases in AMF-inoculated trees, future studies should assess the viability of using AMF inoculation as an alternative to N-fertilization in SRC crops grown on marginal land.

Fertilization with N clearly enhanced growth and biomass to a much greater extent than AMF, and measured N exchange rates in the soil were below detection limits (Chapters 3 and 4), indicating that all the added N had been taken up by the plants.

However, it is important to note that drought and flood only reduced biomass or growth in N-fertilized trees, whereas biomass and growth of unfertilized trees were unaffected. Drought reduced foliar biomass in N-fertilized trees of both species seven months after the drought treatment was imposed (Chapter 4). Previous work has reported that drought has a negative impact on growth of both species (Tallis et al., 2013; Jia et al., 2020) but I showed that, by affecting foliar biomass during the following growing season, even a short drought could have lasting negative effects on growth and biomass gain in the subsequent year. As the trees were necessarily harvested before the height of the final growing season it was not possible to assess the potential legacy effects of drought for SRC crop yield. However, stem diameter growth was lower in N-fertilized willow trees that had experienced both drought and flood. Collectively, these results indicate that while N fertilization boosts crop yields, the rapid growth and high biomass of fertilized SRC crops could make them more susceptible to extreme weather events such as droughts and floods (Bhattacharyya et al., 2012; Nguyen et al., 2018a; Sánchez-Rodríguez et al., 2019).

5.3 Wider context and conclusions

Studies focusing on the interactions between water availability and nutrient addition on SRC growth and carbon dynamics are limited. In addition, the methods employed vary substantially among studies, making direct comparisons difficult and resulting in different recommendations for crop water and nutrient requirements. However, several studies highlight the importance of nutrient availability (Moscatelli et al., 2008) or water availability (Fischer et al., 2018) on growth or carbon dynamics of SRC. The body of research presented in this thesis shows clear interactions between N-addition, AMF inoculation (Chapters 2 & 3), drought (Chapters 3 & 4) and flood treatments (Chapter 4), which have important implications for the carbon budget and viability of SRC crops under future climate changes. To my knowledge no other studies to date have attempted to assess these complex interactions or to determine how they could alter the carbon benefits of bioenergy crops. Thus, my research provides solid foundations for several new avenues of enquiry, which will provide important insights into SRC crop establishment and management.

While this study has provided a solid foundation to build upon, future work should aim to provide a mechanistic understanding of the interactions between SRC crop management, soil GHG emissions, and climate conditions. Specifically, although I assessed AMF colonisation at the end of the experiments, understanding how the soil microbial community responds to changing conditions could provide insights into associated soil GHG dynamics (Randle-Boggis, Ashton and Helgason, 2018; Khalid, Shaaban and Hu, 2019; Bardgett and Caruso, 2020; Sao, Praise and Watanabe, 2023). This area of research would benefit substantially from molecular methods such as DNA analysis to characterise community composition (Kivlin, Hawkes and Treseder, 2011b) and functional gene analysis to relate shifts in microbial communities to changes in function (Santos-Gonzalez et al., 2007; Kaushal et al., 2014; Nguyen et al., 2018b; Randle-Boggis, Ashton and Helgason, 2018; Khalid, Shaaban and Hu, 2019), which were beyond the scope of my work. In conjunction with this, large-scale trials including detailed analyses of belowground biomass, mycorrhizal colonisation, and different organic matter fractions, as well as GHG measurements, would provide linkages between different ecosystem components and their impact on ecosystem C storage in a field setting.

My research focussed on extreme changes in soil moisture content, but rising global temperatures are also of primary importance for soil GHG emissions (Drewer et al., 2017; Sánchez-Rodríguez et al., 2019; Wu et al., 2020; Silverthorn and Richardson, 2021). In addition, increasing use of nutrient addition to compensate for soil degradation and the increasing intensity and occurrence of extreme weather is boosting N_2O emissions in many economically important crops (Muhr et al., 2008; 2008; Wang et al., 2014a; Drewer et al., 2017; Schindler et al., 2020a; Dlamini et al., 2021). Future field trials should include measurements of N_2O, as well as CO_2 and CH_4 fluxes in SRC crops, and pot experiments like those described in this thesis could be used to determine the effects of rising temperatures. Future research avenues should include a long-term study with a minimum of five years of data collection to cover a full crop rotation of between three and five years (DEFRA, 2019) and provide a life-cycle assessment of SRC crops under different climate change scenarios. For example, long-term field trials could incorporate repeated intensive droughts or floods and assess the legacy effects of these events over several growing seasons. Current climate change projections could be used to inform the severity and frequency of the drought and flood events to inform strategies for climate change mitigation (Matsueda and Palmer, 2011.; Gebremichael, Osborne and Orr, 2017; Dodd et al., 2021). A greater understanding of how SRC crop species differ in their responses to extreme climate conditions and insights into the role of AMF communities in improving crop nutrition and resilience would enable the development of site-specific recommendations for growers and inform mitigation measures against extreme weather events, while reducing GHG emissions and maintaining crop yield.

Alongside a more detailed understanding of crop emissions and yield, this body of research is an excellent foundation for considering more detailed GHG emissions calculations especially when considering future policy. The UK and devolved governments are promoting a range of practices on agricultural land to meet Net Zero Carbon targets. However, the calculations used to assess national carbon budgets do not include a detailed breakdown of soil inputs into CO_2 emissions. Nor do they consider the impact of nutrient additions and changes in abiotic conditions on carbon and nutrient cycling in soils. This research highlights that assuming carbon neutrality of SRC crops regardless of climate conditions or nutrient inputs would be unwise, and targeted life cycle assessments under different conditions are required to estimate the potential contribution of SRC crops to net zero targets in future.

Overall, the body of research presented in this thesis demonstrates that interactions between nutrient amendments and extreme changes in soil moisture content with flooding or drought are of substantial importance in determining the true carbon sequestration capacity of SRC crops and should therefore be considered by land-managers and policy-makers aiming to use SRC crops as part of the national strategy to meet Net Zero targets.

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Appendices

Appendix A – Supplementary Materials to Chapter 2

In Chapter 2, I identified the relative benefits of fertilisation and mycorrhizal inoculation during establishment and early development of two short-rotation coppice (SRC) crop species (willow and poplar). I assessed the effects of nutrient addition, inoculation with arbuscular mycorrhizal fungi (AMF) and their interaction on stem height and diameter growth (Table A1), soil organic carbon (SOC) (Table A2) and soil respiration (CO_2 efflux; Table A3) using linear models compared to a corresponding null model using likelihood ratio tests.

Table A 1. *Model results from linear models testing the effects of fertilization with nitrogen (N) or phosphorus (P), inoculation with arbuscular mycorrhizal fungi (AMF) and their interactions on relative height and diameter growth and biomass measurements of willow and poplar over one growing season. Separate analyses were conducted for each tree species; the initial (full) model is shown and the statistics for treatment effect, error term and likelihood ratio test of the final model are given.*

Willow		
Initial model: **Tree diameter** ~ N * P * AMF + block		
Final model: **Tree diameter** ~ N + block *F* = 22.45, Adjusted R^2 = 0.3, *p* = <0.001		
Factor	***t*-value**	***p*-value**
N	5.550	<0.001
Error term Block	-3.755	<0.001
Final model compared with null model	***AIC***	***p*-value**
	-133.247	<0.001
Initial model: **Tree height** ~ N * P * AMF + block		
Final model: **Tree height** ~ N + block F = 1.87, Adjusted R^2 = 0.017, p = 0.168		
Factor	***t-value***	***p-value***
N	-1.326	0.188
Error term		
Block	-1.368	0.174
Final model compared with null model	***AIC***	***p-value***
	8.75	0.168
Poplar		
Initial model: **Tree diameter** ~ N * P * AMF + block		
Final model: **Tree diameter** ~ N + block F = 9.82, Adjusted R^2 = 0.158, p = <0.001		
Factor	***t-value***	***p-value***
N	4.413	<0.001
Error term		
Block	-0.337	0.737
Final model compared with null model	***AIC***	***p-value***
	-144.122	<0.001
Initial model: **Tree height** ~ N * P * AMF + block		
Final model: **Tree height** ~ N + block F = 8.376, Adjusted R^2 = 0.1408, p = <0.001		
Factor	***t-value***	***p-value***
N	3.888	<0.001
Error term		
Block	-1.293	0.199
Final model compared with null model	***AIC***	***p-value***
	51.832	<0.001
Leaf mass		
Initial model: **Leaf** ~ N * P * AMF + species		
Final model: **Leaf** ~ N + species F = 53.71, Adjusted R^2 = 0.772, p = <0.001		
Factor	***t-value***	***p-value***
N	10.34	<0.001
Species	0.73	0.471
Final model compared with null model	***AIC***	***p-value***
	15.065	<0.001
Woody biomass		

Initial model: **Aboveground biomass** ~ N * P * AMF + species		
Final model: **Aboveground biomass** ~ N * AMF + species F = 9.559, Adjusted R^2 = 0.5248, p = <0.001		
Factor	***t-value***	***p-value***
N	5.16	<0.001
AMF	1.651	0.11
Species	-2.186	0.037
N * AMF	-1.834	0.077
Final model compared with null model	***AIC***	***p-value***
	260.368	<0.001
Root biomass		
Initial model: **Belowground biomass** ~ N * P * AMF + species		
Final model: **Belowground biomass** ~ N + species F = 49.82, Adjusted R^2 = 0.759, p = <0.001		
Factor	***t-value***	***p-value***
N	9.979	<0.001
Species	0.234	0.817
Final model compared with null model	***AIC***	***p-value***
	60.255	<0.001
Total biomass		
Initial model: **Total biomass** ~ N * P * AMF + species		
Final model: **Total biomass** ~ N * AMF + species F = 26.67, Adjusted R^2 = 0.768, p = <0.001		
Factor	***t-value***	***p-value***
N	8.413	<0.001
AMF	2.259	0.032
Species	-0.959	0.346
N * AMF	-1.874	0.072
Final model compared with null model	***AIC***	***p-value***
	87.941	<0.001

Table A 2. *Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N) or inoculation with arbuscular mycorrhizal fungi (AMF) and their interactions on soil total organic carbon (TOC) under willow and poplar over two growing seasons. Separate analyses were conducted for each tree species; the initial (full) model is shown model is shown and the statistics for treatment effect, error term and likelihood ratio test of the final model are given.*

Willow		
Initial model: **TOC** ~ N*AMF		
Final model: **TOC** ~ N + AMF F = 0.1933, Adjusted R-squared = -0.026, p = 0.8247		
Fixed effects	***t-value***	***p-value***
N	-0.569	0.572
AMF	0.251	0.803
Final model compared with null model	***AIC***	***p-value***
	480.9247	0.8247
Poplar		
Initial model: **TOC** ~ N*AMF Final model: **TOC** ~ AMF F = 4.958, Adjusted R-squared = 0.05657, p = 0.0294		
Fixed effects	***t-value***	***p-value***
AMF	-2.227	0.0294
Final model compared with null model	***AIC***	***p-value***
	458.6836	0.02945

Table A 3. *Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N) or phosphorus (P), inoculation with arbuscular mycorrhizal fungi (AMF) and their interactions on soil respiration (Soil CO2) under willow and poplar over two growing seasons. Separate analyses were conducted for each tree species; the initial (full) model is shown and the statistics for the fixed effects and likelihood ratio test of the final model are given.*

Willow			
Initial model: **Soil CO2** ~ N * P * AMF + (1|block) + (1|n.date)			
Final model: **Soil CO2** ~ N + (1|block) + (1|n.date)			
Fixed effects	***Coefficient***	***t-value***	***p-value***
N	0.53	3.037	<0.001
Final model compared with null model	χ^2	***AIC***	***p-value***
	35.34	1857.335	<0.001
poplar			
Initial model: **Soil CO2** ~ N * P * AMF + (1|block) + (1|n.date)			
Final model: **Soil CO2** ~ N * AMF + (1|block) + (1|n.date)			
Fixed effects	***Coefficient***	***t-value***	***p-value***
N	0.21	6.682	<0.001
N + AMF	-0.16	5.56	<0.001
AMF * N	0.17	-3.467	<0.001
Final model compared with null model	χ^2	***AIC***	***p-value***
	62.67	203.36	<0.001

Appendix B – Supplementary Materials to Chapter 3

In Chapter 3, I assessed how fertilisation and AMF inoculation influence on soil respiration, and nutrient exchange rates under the SRC crop species willow and poplar during recovery from an intense drought event. I assessed the success of mycorrhizal colonisation (Table B1). I assessed the influence of N-addition, P-addition, AMF inoculation and their interaction on soil water content on the final day of drought (Table B2), soil water content on 28-day recovery period following a rewetting period (Table B3), respiration rates (Table B4), peak CO_2 efflux during rewetting (Table B5), and nutrient exchange rates after recovery from drought (Table B6) using linear mixed effects models. The final models were tested against a corresponding null model using likelihood ratio tests.

Table B 1. *Results of Wilcoxon rank sum tests comparing AMF inoculated and uninoculated soils under willow and poplar trees.*

Willow	
Response variable	***p-value***
Arbuscles	<0.001
Hyphae	0.002
Proportion of AMF	<0.001
Poplar	
Response variable	***p-value***
Arbuscles	0.003
Hyphae	0.062
Proportion of AMF	0.002

Table B 2. *Model results from linear models testing the effects of drought or fertilization with nitrogen (N) and their interactions on soil water content (swc). Separate analyses were conducted for each tree species; the initial (full) model is shown and the statistics for treatment effect, error term and likelihood ratio test of the final model are given.*

Willow		
Initial model: **swc** ~ drought * N		
Final model: **swc** ~ drought * N F = 102.3, Adjusted R-squared = 0.7628, p = <0.001		
Explanatory factor	***t-value***	***p-value***
Drought	-10.477	<0.001
N	-3.022	0.00369
Drought * N	0.926	0.35812
Final model compared with null	***AIC***	***p-value***
	291.9619	<0.001
Poplar		
Initial model: **swc** ~ drought * N		
Final model: **swc** ~ drought * N F = 120.8, Adjusted R-squared = 0.8508, p = <0.001		
Explanatory factor	***t-value***	***p-value***
Drought	-7.521	<0.001
N	-3.735	<0.001
Drought * N	-4.79	<0.001
Final model compared with null	***AIC***	***p-value***
	228.0382	<0.001

Table B 3: *Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N) or inoculation with arbuscular mycorrhizal fungi (AMF), drought and their interactions on soil water content (swc) during the 28 days following rewetting under willow and poplar. Separate analyses were conducted for each tree species; the initial (full) model is shown and the statistics for the fixed effects and likelihood ratio test of the final model are given.*

Willow			
Initial model: **swc**~ drought * N + block + days			
Final model: **swc~** drought * N + block +days			
Explanatory factor	**Coefficient**	**t-value**	**p-value**
Drought	-3.777094	-14.197	<0.001
N	-2.305764	-8.719	<0.001
Drought * N	1.419482	3.784	<0.001
Final model compared with null model	**χ^2**	**AIC**	**p-value**
	276.82	2227.094	<0.001
Poplar			
Initial model: **swc**~ drought * N + block + days			
Final model: **swc~** drought + N + block +days			
Explanatory factor	**Coefficient**	**t-value**	**p-value**
Drought	-1.509942	-10.83	<0.001
N	-1.412513	-10.13	<0.001
Final model compared with null model	**χ^2**	**AIC**	**p-value**
	189.09	1916.979	<0.001

***Table B 4.** Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N) or phosphorus (P), inoculation with arbuscular mycorrhizal fungi (AMF), drought and their interactions on soil CO2 efflux under willow and poplar. Separate analyses were conducted for each tree species; the initial (full) model is shown and the statistics for the fixed effects and likelihood ratio test of the final model are given.*

Willow			
Initial model: **Soil CO2**~ drought * N * P * AMF + block + date Final model: **Soil CO2**~ drought * N * P * AMF + block + date			
Explanatory factor	**Coefficient**	**t-value**	**p-value**
Drought	-0.12	-1.127	0.260
AMF	-0.36	-3.555	0.000
N	0.45	4.448	0.000
P	-0.11	-1.119	0.264
Drought * AMF	0.95	6.563	<0.001
Drought * N	0.11	0.754	0.451
Drought * P	0.24	1.650	0.100
AMF * N	0.39	2.713	0.007
AMF * P	0.37	2.572	0.010
N * P	0.31	2.163	0.031
Drought * AMF * N	-0.91	-4.441	<0.001
Drought * AMF * P	-0.75	-3.658	0.000
Drought * N * P	-0.5	-2.433	0.015
AMF * N * P	-0.62	-3.063	0.002
Drought * AMF * N * P	0.99	3.461	<0.001
Final model compared with null model	χ^2	**AIC**	**p-value**
	212.79	606.4	<0.001
Poplar			
Initial model: **Soil CO2**~ drought * N * P * AMF + block + date			
Final model: **Soil CO2**~ * AMF + N + P + N * P + block + date			
Explanatory factor	**Coefficient**	**t-value**	**p-value**
AMF	0.17	5.294	<0.001
N	0.47	7.958	<0.001
P	-0.35	-4.579	<0.001
N * P	0.32	2.672	0.008
Final model compared with null model	χ^2	**AIC**	**p-value**
	200.1	793.8	<0.001

Table B 5. *Model results from linear models assessing the effects of fertilization with nitrogen (N) or phosphorus (P), inoculation with arbuscular mycorrhizal fungi (AMF) and their interactions on peak soil CO_2 efflux during rewetting after a drought event. Separate analyses were conducted for each tree species; the initial (full) model is shown and the statistics for the treatment effect and likelihood ratio test of the final model are given.*

Willow		
Initial model: **Rewetting peak flux** ~ AMF * nutrient		
Final model: **Rewetting peak flux** ~ nutrient F = 2.839, Adjusted R^2 = 0.08053, p = 0.045		
Factor	***t-value***	***p-value***
N + P	-3.725	<0.001
N	6.057	<0.001
P	-2.745	0.008
Final model compared with null model	***AIC***	***p-value***
	2.88.833	0.045
Poplar		
Initial model: **Rewetting peak flux** ~ AMF * nutrient Final model: **Rewetting peak flux** ~ nutrient F = 7.798, Adjusted R^2 = 0.2445, p = <0.001		
Factor	***t-value***	***p-value***
N + P	1.558	0.125
N	2.539	0.014
P	-1.958	0.052
Final model compared with null model	***AIC***	***p-value***
	278.625	<0.001

Table B 6. *Model results from linear models assessing the effects of fertilization with nitrogen (N) or phosphorus (P), inoculation with arbuscular mycorrhizal fungi (AMF), drought and their interactions on nutrient availability (measured as ion exchange rates) under willow and poplar. Separate analyses were conducted for each tree species and nutrient; the initial (full) model is shown and the statistics for the treatment effect and likelihood ratio test of the final model are given.*

Willow		
Initial model: **Phosphorus** ~ AMF * N * P * drought		
Final model: **Phosphorus** ~ N + P + drought F = 19.37, Adjusted R^2 = 0.4666, p = <0.001		
Factor	***t-value***	***p-value***
N	-3.725	<0.001
P	6.057	<0.001
Drought	-2.745	0.008
Final model compared with null model	***AIC***	***p-value***
	517.628	<0.001
Initial model: **Potassium** ~ AMF * N * P * drought		
Final model: **Potassium** ~ N + P + drought F = 2.908, Adjusted R^2 = 0.08328, p = 0.042		
Factor	***t-value***	***p-value***
N	2.263	0.027
P	1.688	0.096
Drought	0.867	0.389
Final model compared with null model	***AIC***	***p-value***
	857.228	0.042
Initial model: **Calcium** ~ AMF * N * P * drought		
Final model: **Calcium** ~ N + P + drought F = 15.71, Adjusted R^2 = 0.4119, p = <0.001		
Factor	***t-value***	***p-value***
N	-5.568	<0.001
P	-0.211	0.834
Drought	-4.009	<0.001
Final model compared with null model	***AIC***	***p-value***
	944.926	<0.001
Initial model: **Magnesium** ~ AMF * N * P * drought		
Final model: **Magnesium** ~ N + P + drought F = 10.86, Adjusted R^2 = 0.3196, p = <0.001		
Factor	***t-value***	***p-value***
N	-4.207	<0.001
P	0.946	0.348
Drought	-3.741	<0.001
Final model compared with null model	***AIC***	***p-value***
	654.483	<0.001
Poplar		
Initial model: Phosphorus ~ AMF * N * P * drought Final model: Phosphorus ~ N + P + drought F = 25.29, Adjusted R^2 = 0.5363, p = <0.001		
Factor	***t-value***	***p-value***
N	-4.662	<0.001
P	7.480	<0.001
Drought	-0.770	0.444
Final model compared with null model	***AIC***	***p-value***

	551.758	<0.001
Initial model: Potassium ~ AMF * N * P * drought Final model: Potassium ~ N + P + drought F = 4.286, Adjusted R² = 0.1353, p = 0.008		
Factor	***t-value***	***p-value***
N	0.281	0.779
P	3.498	<0.001
Drought	0.575	0.567
Final model compared with null model	***AIC***	***p-value***
	876.989	0.001
Initial model: Calcium ~ AMF * N * P * drought Final model: Calcium ~ N + P + drought F = 4.31, Adjusted R² = 0.1362, p = <0.008		
Factor	***t-value***	***p-value***
N	-3.465	<0.001
P	-0.063	0.950
Drought	-0.944	0.349
Final model compared with null model	***AIC***	***p-value***
	953.648	0.008
Initial model: Magnesium ~ AMF * N * P * drought Final model: Magnesium ~ N + P + drought F = 3.93, Adjusted R² = 0.1224, p = 0.1224		
Factor	***t-value***	***p-value***
N	-3.390	0.001
P	-0.214	0.831
Drought	-0.433	0.667
Final model compared with null model	***AIC***	***p-value***
	633.15	0.013

Appendix C – Supplementary Materials to Chapter 4

In Chapter 4, I assessed the influence of fertilisation, drought history and flooding on soil CO_2 (Table C1) and methane (CH_4) fluxes (Table C2), nutrient availability (Table C3), nutrient concentrations in leaf and soil (Table C4), foliar biomass, stem height, stem diameter and stem biomass (Table C5). AMF inoculation treatments were spread equally over flooded and previously droughted treatments but were confounded with N-addition. Thus, the experimental design was factorial with two levels for each treatment (flooded vs. not flooded, droughted vs. non-droughted and with N vs. no added N). I used linear mixed effects models with N-fertilisation, drought history, flooding and their interactions as fixed effects, and block and time as random effects. The final models were tested against a corresponding null model using likelihood ratio tests.

Table C 1. *Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N), flooding, drought history, and their interactions on soil* CO_2 *efflux (measured as ion exchange rates) under willow and poplar. Separate analyses were conducted for each tree species and nutrient; the initial (full) model is shown and the statistics for the fixed effects, random effects and likelihood ratio test of the final model are given.*

Willow			
Initial model: **Soil CO2**~ N * water treatment combination + block + days			
Final model: **Soil CO2**~ water treatment combination + N + block + days			
Fixed effects	**Coefficient**	**t-value**	**p-value**
Drought	0.3980547	2.539	0.011
Drought + Flood	1.21573682	7.753	<0.001
Flood	1.14501617	7.302	<0.001
N	0.7518705	4.795	<0.001
Drought + N	-1.0623691	-4.703	<0.001
Drought + Flood + N	-0.029991	-0.135	0.892
Flood + N	-0.4788879	-2.16	0.031
Final model compared with null model	χ^2	**AIC**	**p-value**
	9173.1	3120.2	<0.001
Poplar			
Initial model: **Soil CO2**~ N * water treatment combination + block + days			
Final model: **Soil CO2**~ water treatment combination + N + block + days			
Fixed effects	**Coefficient**	**t-value**	**p-value**
Drought	0.5531604	3.282	0.001
Drought + Flood	0.8419478	4.995	<0.001
Flood	0.2603745	1.545	0.123
N	0.373692	2.217	0.027
Drought + N	-0.1117579	-0.477	0.633
Drought + Flood + N	0.2844441	1.215	0.225
Flood + N	1.6706509	7.134	<0.001
Final model compared with null model	χ^2	**AIC**	**p-value**
	9093	3183	<0.001

Table C 2. *Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N), flooding, drought history, and their interactions on soil CH$_4$ fluxes under willow and poplar. Separate analyses were conducted for each tree species and nutrient; the initial (full) model is shown and the statistics for the fixed effects, random effects and likelihood ratio test of the final model are given.*

Willow			
Initial model: **Soil CH4**~ N * water treatment combination + block + days			
Final model: **Soil CH4**~ water treatment combination + N + block + days			
Fixed effects	**Coefficient**	**t-value**	**p-value**
Drought	-0.2062737	-0.666	0.505
Drought + Flood	3.26250731	10.538	<0.001
Flood	3.02344549	9.766	<0.001
N	-0.353387	-1.141	0.254
Drought + N	0.59745937	1.34	0.18
Drought + Flood + N	-0.0707615	-0.162	0.872
Flood + N	1.55991021	3.563	<0.001
Final model compared with null model	χ^2	**AIC**	**p-value**
	416.5	4400.2	<0.001
Poplar			
Initial model: **Soil CO2**~ N * water treatment combination + block + days			
Final model: **Soil CO2**~ water treatment combination + N + block + days			
Fixed effects	**Coefficient**	**t-value**	**p-value**
Drought	0.536184	-2.716	0.006
Drought + Flood	-4.2620355	8.446	<0.001
Flood	2.0850845	9.85	<0.001
N	0.5008333	-2.579	0.01
Drought + N	-2.5598055	1.382	0.167
Drought + Flood + N	-2.3363968	5.787	<0.001
Flood + N	1.8264177	0.961	0.337
Final model compared with null model	χ^2	**AIC**	**p-value**
	529.8	4731.9	<0.001

Table C 3. *Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N), flooding, drought history, and their interactions on nutrient availability (measured as ion exchange rates) under willow and poplar. Separate analyses were conducted for each tree species and nutrient; the initial (full) model is shown and the statistics for the fixed effects, random effects and likelihood ratio test of the final model are given.*

Willow		
Initial model: **Potassium** ~ N * flood * drought		
Final model: **Potassium** ~ N + 1 F = 31.51, Adjusted R^2 = 0.329, p = <0.001		
Factor	***t-value***	***p-value***
N	5.613	<0.001
Final model compared with null model	***AIC***	***p-value***
	103.05	<0.001
Initial model: **Calcium** ~ N * flood * drought		
Final model: **Calcium** ~ flood + N + flood * N +1 F = 3.816, Adjusted R^2 = 0.1199, p = 0.0144		
Factor	***t-value***	***p-value***
Flood	-3.196	0.002
N	-2.298	0.251
Flood * N	3.016	0.004
Final model compared with null model	***AIC***	***p-value***
	43.15	0.144
Poplar		
Initial model: **Potassium** ~ N * flood * drought		
Final model: **Potassium** ~ flood + N + flood * N +1 F = 5.425, Adjusted R^2 = 0.176, p = <0.002		
Factor	***t-value***	***p-value***
Flood	0.166	0.868
N	3.557	<0.001
Flood * N	-2.049	0.450
Final model compared with null model	***AIC***	***p-value***
	64.96	0.002
Initial model: **Calcium** ~ N * flood * drought		
Final model: **Calcium** ~ drought + flood + drought * flood + 1 F = 35.181, Adjusted R^2 = 0.1683, p = 0.003		
Factor	***t-value***	***p-value***
Drought	0.28	0.781
Flood	-0.207	0.836
Drought * flood	-2.329	0.023
Final model compared with null model	***AIC***	***p-value***
	39.91	0.003
Initial model: **Magnesium** ~ N * flood * drought		
Final model: **Magnesium** ~ drought + flood + drought * flood + 1 F = 35.181, Adjusted R^2 = 0.1683, p = 0.003		
factor	***t-value***	***p-value***
Drought	-0.729	0.469
Flood	-0.139	0.890

Drought * flood	-1.845	0.070
Final model compared with null model	***AIC***	***p-value***
	27.6	0.003

Table C 4. *Model results from linear models testing the effects of fertilization with nitrogen (N), flooding, drought history, and their interactions on soil C, soil N, soil C:N ratio, foliar C (leaf C), leaf N, leaf C:N ratio and soil ph. Separate analyses were conducted for each tree species and nutrient; the initial (full) model is shown and the statistics for the treatment effects and likelihood ratio test of the final model are given.*

Willow		
Initial model: **Soil C** ~ combined water treatment * N Final model: **Soil C** ~ combined water treatment * N F = 1.614, Adjusted R-squared = 0.06388, p = 0.1504		
Factor	**t-value**	**p-value**
Drought	0.172	0.864
Flood	-0.252	0.802
Flood + drought	-0.644	0.522
N	-0.064	0.949
Drought * N	1.599	0.115
Flood * N	0.196	0.845
Flood + drought * N	0.246	0.807
Final model compared with null model	**AIC**	**p-value**
	159.9	0.1504
Initial model: **Soil N** ~ combined water treatment * N Final model: **Soil N** ~ combined water treatment * N F = 1.588, Adjusted R-squared = 0.06133, p = 0.158		
Factor	**t-value**	**p-value**
Drought	0.664	0.510
Flood	0.502	0.618
Flood + drought	0.395	0.695
N	-0.054	0.957
Drought * N	1.382	0.173
Flood * N	-0.533	0.596
Flood + drought * N	-0.379	0.706
Final model compared with null model	**AIC**	**p-value**
	-61.14829	0.158
Initial model: **Soil C: N** ~ combined water treatment * N Final model: **Soil C: N** ~ combined water treatment * N F = 0.7816, Adjusted R-squared = 0.02487, p = 0.6054		
Factor	**t-value**	**p-value**
Drought	-0.884	0.380
Flood	-0.010	0.317
Flood + drought	-0.946	0.843
N	0.200	0.744
Drought * N	-0.328	0.406
Flood * N	0.837	0.578
Flood + drought * N	0.560	0.578
Final model compared with null model	**AIC**	**p-value**
	464.0849	0.6054

Initial model: **leaf C** ~ combined water treatment * N Final model: **leaf C** ~ combined water treatment * N F = 0.8691, Adjusted R-squared = -0.015 p = <0.5366		
Factor	**t-value**	**p-value**
Drought	0.645	0.5215
Flood	0.471	0.6397
Flood + drought	0.9	0.3721
N	1.078	0.2855
Drought * N	-1.963	0.0548
Flood * N	-0.55	0.5844
Flood + drought * N	-1.123	0.2663
Final model compared with null model	**AIC**	**p-value**
	352.0385	0.5366
Initial model: **leaf N** ~ combined water treatment * N Final model: **leaf N** ~ combined water treatment * N F = 4.161, Adjusted R-squared = 0.263, p = <0.001		
Factor	**t-value**	**p-value**
Drought	1.262	0.212
Flood	1.679	0.1
Flood + drought	1.474	0.146
N	0.233	0.816
Drought * N	2.018	0.048
Flood * N	-0.768	0.445
Flood + drought * N	1.052	0.297
Final model compared with null model	**AIC**	**p-value**
	57.548	<0.001
Initial model: **leaf C: N** ~ combined water treatment * N Final model: **leaf C: N** ~ combined water treatment * N F = 3.752, Adjusted R-squared = 0.2371, p = 0.002		
Factor	**t-value**	**p-value**
Drought	-1.707	0.094
Flood	-2.155	0.036
Flood + drought	-1.768	0.083
N	-0.245	0.808
Drought * N	-1.372	0.176
Flood * N	1.125	0.265
Flood + drought * N	-0.959	0.342
Final model compared with null model	**AIC**	**p-value**
	408.1666	0.002

Initial model: **Soil pH** ~ combined water treatment * N Final model: **Soil pH** ~ combined water treatment * N F = 0.8246, Adjusted R-squared = -0.01966, p = 0.5697		
Factor	**t-value**	**p-value**
Drought	-1.228	0.225
Flood	-0.862	0.392
Flood + drought	0.692	0.492
N	-0.444	0.659
Drought * N	0.721	0.474
Flood * N	0.055	0.956
Flood + drought * N	-0.648	0.519
Final model compared with null model	**AIC**	**p-value**
	-20.57097	0.5697
Poplar		
Initial model: **Soil C** ~ combined water treatment * N Final model: **Soil C** ~ combined water treatment * N F = 1.036, Adjusted R-squared = 0.00402, p = 0.4172		
Factor	**t-value**	**p-value**
Drought	-0.860	0.394
Flood	-0.386	0.701
Flood + drought	-1.159	0.252
N	0.544	0.589
Drought * N	0.041	0.967
Flood * N	0.588	0.559
Flood + drought * N	0.328	0.744
Final model compared with null model	**AIC**	**p-value**
	52.002	0.4172
Initial model: **Soil N** ~ combined water treatment * N Final model: **Soil N** ~ combined water treatment * N F = 0.4847, Adjusted R-squared = -0.0618, p = 0.842		
Factor	**t-value**	**p-value**
Drought	0.812	0.420
Flood	0.622	0.536
Flood + drought	1.636	0.107
N	0.717	0.476
Drought * N	-0.482	0.632
Flood * N	-0.741	0.461
Flood + drought * N	-0.961	0.341
Final model compared with null model	**AIC**	**p-value**
	-126.868	0.842
Initial model: **Soil C: N** ~ combined water treatment * N Final model: **Soil C: N** ~ combined water treatment * N F = 0.847, Adjusted R-squared = -0.0176, p = 0.554		
Factor	**t-value**	**p-value**

Drought	-0.920	0.362
Flood	-0.674	0.503
Flood + drought	-2.078	0.042
N	-0.587	0.560
Drought * N	0.419	0.677
Flood * N	0.800	0.427
Flood + drought * N	1.163	0.250
Final model compared with null model	**AIC**	**p-value**
	432.251	0.554
Initial model: **leaf C** ~ combined water treatment * N Final model: **leaf C** ~ combined water treatment * N F = 0.6452, Adjusted R-squared = -0.042 p = 0.7166		
Factor	**t-value**	**p-value**
Drought	-0.687	0.495
Flood	-0.034	0.973
Flood + drought	-0.105	0.917
N	-0.058	0.954
Drought * N	1.435	0.157
Flood * N	0.139	0.89
Flood + drought * N	0.116	0.908
Final model compared with null model	**AIC**	**p-value**
	232.274	0.7166
Initial model: **leaf N** ~ combined water treatment * N Final model: **leaf N** ~ combined water treatment * N F = 14.17, Adjusted R-squared = 0.598, p = <0.001		
Factor	**t-value**	**p-value**
Drought	-0.112	0.912
Flood	1.119	0.268
Flood + drought	0.854	0.397
N	-0.668	0.507
Drought * N	5.173	<0.001
Flood * N	0.135	0.893
Flood + drought * N	3.317	0.002
Final model compared with null model	**AIC**	**p-value**
	36.543	<0.001
Initial model: **leaf C: N** ~ combined water treatment * N Final model: **leaf C: N** ~ combined water treatment * N F = 12.27, Adjusted R-squared = 0.5599, p = <0.001		
Factor	**t-value**	**p-value**
Drought	0.194	0.847
Flood	-1.400	0.167
Flood + drought	-1.009	0.317
N	1.178	0.244
Drought * N	-4.871	<0.001
Flood * N	0.301	0.745
Flood + drought * N	-3.400	0.001
Final model compared with null model	**AIC**	**p-value**

	384.3235	<0.001
Initial model: **Soil pH** ~ combined water treatment * N		
Final model: **Soil pH** ~ combined water treatment * N F = 0.311, Adjusted R-squared = -0843, p = 0.946		
Factor	**t-value**	**p-value**
Drought	-0.296	0.768
Flood	0.473	0.638
Flood + drought	-0.167	0.868
N	-0.266	0.791
Drought * N	0.810	0.421
Flood * N	-0.491	0.625
Flood + drought * N	0.404	0.688
Final model compared with null model	**AIC**	**p-value**
	-68.42331	0.946

Table C 5. Model results from linear mixed effects models testing the effects of fertilization with nitrogen (N), flooding, drought history, and their interactions leaf biomass, steam height, stem diameter and stem biomass. Separate analyses were conducted for each tree species and nutrient; the initial (full) model is shown and the statistics for the fixed effects, random effects and likelihood ratio test of the final model are given.

Willow		
Initial model: **leaf biomass** ~ combined water treatment * N		
Final model: **leaf biomass** ~ combined water treatment * N F = 13.91, Adjusted R-squared = 0.5932, p = <0.001		
Factor	**t-value**	**p-value**
Drought	0.523	0.603
Flood	-0.708	0.482
Flood + drought	-0.932	0.355
N	5.682	<0.001
Drought * N	-4.635	<0.001
Flood * N	0.043	0.966
Flood + drought * N	-3.341	0.002
Final model compared with null model	**AIC**	**p-value**
	364.091	<0.001
Initial model: **stem height** ~ combined water treatment * N		
Final model: **stem height** ~ combined water treatment * N F = 0.6419, Adjusted R-squared = 0.0414, p = 0.7194		
Factor	**t-value**	**p-value**
Drought	0.256	0.799
Flood	0.256	0.799
Flood + drought	-0.844	0.402
N	0.484	0.631
Drought * N	-0.913	0.365
Flood * N	-0.945	0.349
Flood + drought * N	-0.431	0.668
Final model compared with null model	**AIC**	**p-value**
	904.631	0.7194
Initial model: **stem diameter** ~ combined water treatment * N		
Final model: **stem diameter** ~ combined water treatment * N F = 33.29, Adjusted R-squared = 0.782, p = <0.00		
Factor	**t-value**	**p-value**
Drought	0.127	0.899
Flood	0.940	0.351
Flood + drought	2.113	0.039
N	8.581	<0.001
Drought * N	-0.061	0.952
Flood * N	-0.635	0.528
Flood + drought * N	-2.274	0.027
Final model compared with null model	**AIC**	**p-value**
	278.564	<0.001
Initial model: **stem biomass** ~ combined water treatment * N		
Final model: **stem biomass ~** N F = 718.5, Adjusted R-squared = 0.9193, p = <0.001		
Factor	**t-value**	**p-value**
N	26.800	<0.001
Final model compared with null model	**AIC**	**p-value**

	631.299	<0.001
Poplar		
Initial model: **leaf biomass** ~ combined water treatment * N		
Final model: **leaf biomass** ~ combined water treatment * N F = 91.68, Adjusted R-squared = 0.911, p = <0.001		
Factor	**t-value**	**p-value**
Drought	0.909	0.367
Flood	-0.338	0.736
Flood + drought	-0.447	0.656
N	12.891	<0.001
Drought * N	-10.953	<0.001
Flood * N	1.566	0.123
Flood + drought * N	-8.482	<0.001
Final model compared with null model	**AIC**	**p-value**
	369.676	<0.001
Initial model: **stem height** ~ combined water treatment * N		
Final model: **stem height** ~ N F = 314.5, Adjusted R-squared = 0.835, p = <0.001		
Factor	**t-value**	**p-value**
Drought	17.73	<0.001
Final model compared with null model	**AIC**	**p-value**
	855.188	<0.001
Initial model: **stem diameter** ~ combined water treatment * N		
Final model: **stem diameter** ~ N F = 316, Adjusted R-squared = 0.833, p = <0.001		
Explanatory value	**t-value**	**p-value**
N	17.780	<0.001
Final model compared with null model	**AIC**	**p-value**
	281.014	<0.001
Initial model: **stem biomass** ~ combined water treatment * N		
Final model: **stem biomass ~** N F = 621.8, Adjusted R-squared = 0.9092, p = <0.001		
Explanatory value	**t-value**	**p-value**
N	24.970	<0.001
Final model compared with null model	**AIC**	**p-value**
	600.699	<0.001