$H_2{}^{18}O$ labelled vapour reveals evidence of radial Péclet Effects in some leaves, but not all.

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Abstract

Previous studies demonstrated the importance of pathways of water movement, hydraulic conductance, and leaf hydraulic design on leaf water isotopic enrichment. The current study addresses the relevance of Péclet effects to leaf water isotopic enrichment by assessing spatial variation and comparing bulk leaf water enrichment to Craig-Gordon modelled values. Vapour isotope labelling was also utilised in an experimental first to quantify the carry-forward of mesophyll water isotope compositions to more distal parts of corn (*Zea mays*), oat (*Avena sativa*), sunflower (*Helianthus annuus*) and foxglove (*Digitalis purpurea*) leaves through coupled leaf gas exchange and isoflux measurements. In this study, we found that the Péclet effect was relevant to patterns of leaf water isotopic enrichment for three out of four species tested. The change in $H_2{}^{18}O$ for both monocots highlighted a vascular Péclet effect, while the change in foxglove showed a mesophyll Péclet effect, both of which were found to be relevant for the isotopic enrichment of transpired vapour and leaf water. In contrast, sunflower did not present any evidence of either a vascular or a mesophyll Péclet effect, suggesting a difference in leaf hydraulic design. These findings help us to understand how differences in leaf hydraulic design and anatomy influence the development of gradients in leaf water isotopes. This improved understanding will assist in the most appropriate choice of theoretical models to accurately predict leaf water isotope composition for applications as varied as predicting past climates from tree ring isotopes or selecting water-use efficient genotypes in crop plants.

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Introduction and Literature Review

1.1 Leaf water and its isotope composition

The leaves of terrestrial plants must remain hydrated to accomplish the wide range of biochemical reactions involved in plant metabolism and growth (Evert & Eichhorn, 2013). Leaves are the main sites of the key biochemical process of photosynthesis, so the maintenance of high leaf water content has been under strong selective pressure, resulting in the evolution of several water regulation mechanisms (Taiz & Zeiger, 2015). These regulation mechanisms have been the topic of intense research interest for decades, and many key research methods have emerged to advance our understanding of leaf water relations and how regulation of leaf water use influences the environment (Jones, 2014).

Leaf water and its oxygen isotope composition are of interest and significance across science, particularly plant physiology, as leaf water influences a wide range of processes within the plant and its environment, thus creating opportunities for many important applications.

Stable isotopes such as oxygen-18 (^{18}O) provide a powerful tool for investigating the carbon and water cycles across various scientific fields. The oxygen isotope composition of leaf water ($\delta^{18}O_{lw}$) influences that of both molecular oxygen ($\delta^{18}O_{O2}$) and carbon dioxide ($\delta^{18}O_{CO2}$). The isotopic composition of leaf water can then be applied for studies of productivity, such as terrestrial versus oceanic, as seen in the Dole Effect, and to help constrain global carbon models (Dole et al. 1954, Bender et al. 1994 as cited in Barbour et al., 2021). As the isotope composition of leaf water influences atmospheric carbon dioxide levels through photosynthesis and respiration, it can be used for a wide range of applications such as interpreting the effects of pollution, resource utilisation and assessing increases in primary production (Saurer et al. 2001, Cernusak et al. 2004 as cited in Farquhar & Cernusak, 2005). Leaf water also imparts an isotopic signal on plant organic material, which can be used to reconstruct past climates such as tree rings (Libby et al., 1976). The study of stable isotopes in water can also be used to track water through systems such as aquifers and continental water recycling and to identify sources of plant water uptake (Aemisegger et al., 2014; Darling et al., 2003; Dawson & Ehleringer, 1991).

There remain significant knowledge gaps in our understanding of leaf water, and stable isotopic techniques show some promise as tools to fill these gaps. Specifically, $\delta^{18}O_{lw}$ has been used to help provide insight into the different pathways of water movement, such as outside xylem transport, and how variation in leaf anatomy and pathways can influence leaf hydraulic conductance (Barbour & Farquhar, 2004; Barbour et al., 2017; Barbour et al., 2021). Studying and modelling the variation in leaf anatomy can help identify areas of uncertainty, such as sites of evaporation in leaves, and ultimately improve understanding for future research and applications (Holloway-Phillips et al., 2016). Brodribb et al. (2013) investigated the diversity of leaf anatomy both functionally and evolutionarily, its effect on hydraulic architecture, such as the need for gas exchange and pathways for water transport to different tissues such as the mesophyll. Other studies have also examined how gradients in leaf water isotope composition can vary with different pathways of water transport and may result in enrichment through modelling techniques (Barbour & Farquhar, 2004).

The oxygen isotope composition of leaf water has been utilised to assess plants' physiological and genetic changes, such as stomatal conductance and crop yield (Barbour et al., 2000; Farquhar & Cernusak, 2005). Furthermore, the oxygen isotope enrichment of leaf water is significant within plant physiology as it provides a record of important parameters, such as leaf evaporative conditions, along with crucial interactions and responses of plants to the environment (Barbour & Farquhar, 2004; Barbour, 2007). Environmental variables such as temperature, relative humidity, wind, and light can affect rates of water transport and levels of enrichment as they influence stomatal conductance and gas phase transport (Flanagan et al., 1991). The oxygen isotope composition of plant tissues reflects water taken up by the plant and evaporative and diffusional effects during transpiration; it can be applied across ecophysiological studies. Applications include assessing aspects of plant performance such as resource use efficiency, productivity levels of key processes such as photosynthesis and responses to stressors. Furthermore, applications can be extended to regulating plant water loss, breeding for improved water use efficiency and yield in crop species, and identification of the origins of plant tissues, such as the authenticity of products and forensic analysis (Barbour, 2007; Song et al., 2015b).

1.1.1 Pathways of water movement in leaves

Mechanistic understanding of water movement in leaves emerged in the late 1890s with the development of the cohesion-tension theory describing the vertical movement of sap through the xylem due to the negative pressure formed by the liquid to gas phase change and subsequent transpiration from the leaves (Bohm, 1893; Dixon & Joly, 1894). Strugger (1943) extended this theory, describing water movement primarily within the apoplast (a non-living portion of plant tissue, including cell walls). Dainty (1963) developed the idea that the transport of mesophyll water occurs via diffusion as it is required to travel across membranes. Later, following an experiment on transpiration pathways in wheat leaves, transpiration water was suggested to move into the symplasm at the bundle sheath interface (Canny, 1986, 1988, 1990). This mechanism became referred to as symplastic water movement and was supported further by the discovery of aquaporins in plants (Daniels et al., 1994; Höfte et al., 1992)

Steudle et al. (1993) have narrowed water movement through plant tissues to three parallel pathways. The three pathways include symplastic movement through plasmodesmata, transcellular movement across membranes using aquaporins, and apoplastic flow in cell walls. Aquaporins were first discovered in the tonoplast and then in the plasma membrane of plant cells and have since been found to have a significant role in promoting water fluxes (Canny, 1986; Frangne et al., 2001). More recent modelling studies have suggested that water transport may also occur in the gas phase (Buckley et al., 2015; Rockwell et al., 2014).

It is also important to consider the end point of pathways of water movement; however, the exact location of sites of evaporation within plants is strongly debated. Tanton and Crowdy (1972) suggested that most evaporation occurs from cells near the stomata. This finding was further supported by Meidner (1976) and Cowan (1978), who identified that evaporation occurs from guard, subsidiary, and epidermal cells near the stomata. Pesacreta and Hasenstein (1999) discovered the existence of an interior cuticle thought to help prevent water loss, thus limiting evaporation and suggesting that it may occur from mesophyll cells instead. There are three main theories for sites of evaporation within the leaf. Firstly, the intercellular air space refers to evaporation from mesophyll and epidermal cells exposed to the vapour phase. Secondly, substomatal cavities for evaporation from mesophyll and epidermal cells next to the substomatal cavities. Thirdly, the peristomatal region accounts for 75% of the peristomatal region and the rest from mesophyll and epidermal cells near the substomatal cavity. Although it is not known for sure exactly where evaporation occurs in the leaf, it is vital to consider as it affects pathway length for water transport and may influence the development of gradients in $H_2{}^{18}O$ enrichment (Barbour & Farquhar, 2004). Further, spatially-explicit modelling of coupled water and heat transport within leaves (Buckley et al., 2017) suggests that vapour may re-condense within leaves of some plants (specifically, an amphistomatous leaf with a clear distinction between palisade and spongy mesophyll layers). The effects on water isotopes of re-condensation of vapour within leaves remain uncertain.

Finally, a study by Farquhar and Cernusak (2005) highlighted the idea of one-way fluxes, suggesting that under certain conditions, more water can enter the leaf via vapour than liquid phase, i.e., from the atmosphere rather than the soil. The concept of greater vapour than liquid flux can be mathematically described by $g\ (w_i - w_a)$, where g refers to the total conductance, w_i is water vapour inside the leaf, and w_a is water vapour from the atmosphere. Although this contradicts the usual view of plant water being sourced mainly from the soil, it implies that one-way fluxes of vapour from the atmosphere may strongly influence the isotopic composition of leaf water compared to that from the soil (Farquhar & Cernusak, 2005). The influence of the one-way vapour flux on isotopic compositions has been explored through theoretical models by Farquhar et al. (2021) but has not yet been experimentally tested.

1.1.2 Hydraulic conductance

It is important to consider the water movement in liquid and gas phases, as they contribute to hydraulic conductance. Hydraulic conductance is very dynamic and can be influenced by leaf anatomy and age, and environmental differences such as diurnal changes in light and temperature levels, as well as resources and stressors such as water availability (e.g., drought) and nutrients. Hydraulic conductance is essential in plant physiology as it can be used to compare species by assessing differences in anatomy and structure. The differences in anatomy, such as the xylem and mesophyll tissues and veins, are significant as they contribute to the efficiency of water transport and can also affect gas exchange through stomatal conductance. Hydraulic conductance typically refers to the efficiency of water transport within the plant or leaf, such as the different pathways and pools ending with transpiration or evaporation and their potential influences. Leaf hydraulic conductance refers to the ratio of water flow rate (velocity) through the leaf (petiole, veins, bundle sheath and mesophyll) to the water potential gradient which drives flow (Sack & Scoffoni, 2012).

Water potential refers to the potential energy of water, often associated with the various drivers for water transport, such as osmosis, gravity, and different sources of pressure. Water potential can be influenced by variables such as temperature and relative humidity for vapour phase pathways, as it can lead to gradients affecting water movement and contributing to conductance (Jarvis, 1976).

Rockwell et al. (2014) investigated the distribution of evaporation and competition between liquid and vapour phase transport by modelling energy fluxes. Rockwell demonstrated the importance of thermal conduction for liquid and vapour phase transport as it affects water potential and temperature gradients influencing diffusion. As for leaf hydraulic design, Rockwell found that the ratio of airspaces in the palisade and spongy mesophyll affected thermal conductivity and the efficiency of heat conduction or latent heat transport. The number of air spaces in the spongy mesophyll was shown to play a critical role in the evaporation in the leaf's peristomatal or perivascular areas. Influences such as the level of transpiration and temperature gradients were also shown to affect the amount of vapour transport taking place and, thus, the hydraulic efficiency (Rockwell et al., 2014).

Another study by Buckley (2015) aimed to evaluate the contributions of each pathway to hydraulic conductance and how they are affected by gradients and differing conditions. Apoplastic movement of water was found to dominate symplastic transport under most conditions. The remaining conductance was attributed to symplastic and gas (vapour) pathways with vertical conductance varying with temperature gradients between tissue types. The gas phase pathway was found to be significant for vertical transport, while symplastic pathways only had a minor influence. The conductance of the gas phase pathway was seen as likely to increase with inner leaf temperature following light absorption. Other parameters that were noted to influence conductance rates among pathways include the Poiseuille radius of pathways for bulk flow through the apoplast and the thickness of cell walls (Buckley, 2015).

Buckley et al. (2015) further explored how leaf anatomy influences outside xylem water transport and hydraulic conductance. Hydraulic conductance varies greatly among species due to the anatomy of leaves, such as traits seen in the veins and mesophyll, as they can affect water status, flow, and stomatal regulation (Buckley et al., 2015).

Apoplastic transport was again seen to dominate across all tissue types. Vein length per unit of leaf area (VLA) is expected to increase hydraulic conductance both within and outside the xylem. VLA determines the number of parallel pathways for flow throughout the veins, helping to decrease the horizontal path length for transport from minor veins, thus increasing conductance. VLA could also be linked to outside xylem transport through an interaction between size and the permeability of bundle sheath cells and possible bundle sheath extensions. The thickness and ratio of mesophyll tissues, such as spongy to palisade mesophyll, are also linked to hydraulic conductance.

Further support was also shown for increased gas phase transport under vertical temperature gradients. VLA was observed to strongly influence conductance as it helped increase bundle sheath surface area, effectively decreasing horizontal path length. An increase in the spongy mesophyll radius caused a decrease in conductance as the area for available transport declines without an increase in cell wall thickness. However, leaf thickness was found to have little influence, and bundle sheath extensions were noted to increase conductance by 10% in several heterobaric species. VLA, leaf thickness and the distance between vascular bundles and the epidermis all helped to increase conductivity as they reduce path length (Buckley et al., 2015).

Often these traits are connected to outside xylem hydraulic conductance across species and are essential for determining sites of evaporation and resistances. Resistances for outside xylem hydraulic conductance mostly come from the spongy mesophyll and relate to its low connectivity between cells, reducing the available area for liquid transport. Bundle sheaths contribute little resistance to outside xylem conductance, but this requires further investigation (Buckley et al., 2015).

Buckley et al. (2017) investigated the influence of temperature gradients in anisothermal vapour transport (AVT), which was a major driver in the distribution of evaporation. The study examined vapour transport, specifically sites of evaporation in leaves and looked at different end points for pathways such as rehydrating cells during active transpiration (Buckley et al., 2017).

Interpretations of hydraulic conductance across all scales and pathways must consider that the end points may not always be points of evaporation when vapour phase transport is included but are likely close to the stomata such as the mesophyll (Buckley et al., 2017).

Some other key influences were highlighted in a study looking at the different pathways for water movement and enrichment by Barbour & Farquhar (2004). Throughout the models created for analysis: cell size, cell wall thickness, and anatomical variation, such as the presence of aquaporins and potential sites for evaporation, were shown as important factors. Cell size is important as it forms connections for water transport and determines pathways for bulk flow. Usually, connections are quite small, so bulk flow is not possible, making the role of aquaporins significant for water transport between cells. The thickness of cell walls in tissues such as the parenchyma and mesophyll was also found to influence path length as an increase in thickness was predicted to decrease path length for apoplastic water movement. These factors contribute to hydraulic conductance, affecting path length for water movement. However, they are also relevant to the generation of gradients and enrichment, such as in the Péclet effect. Sites of evaporation were also modelled and found to have a significant effect on path length independently, which was affected by the number of cells involved in transport (Barbour & Farquhar, 2004).

1.2 Isotope enrichment in leaves

Oxygen occurs naturally on earth and in its atmosphere in three forms, with ^{16}O being the most abundant, along with ^{17}O and ^{18}O. ^{16}O is the lightest, while ^{17}O and ^{18}O are heavier due to a greater mass resulting from extra neutrons. Water within plant tissues becomes enriched with $H_2{}^{18}O$ during biophysical processes that occur within leaves. Leaf water is often considered to be comprised of different pools, and their level of enrichment can vary based on differences in anatomy and, hence, hydraulic design between species. The difference in concentrations of isotopes seen across pools is due to isotopic fractionation effects such as the kinetic and equilibrium isotope effects which occur during transpiration.

This investigation focuses on variation in the stable oxygen isotope composition of leaf water between species and tests the hypothesis that the one-way flux of vapour into the leaf influences the transpiration isoflux from a more distal leaf portion due to the presence of a radial Péclet effect from the lamina mesophyll to the vascular tissue. A more complete understanding of the variation in leaf water isotopes, and the processes driving this variation, will be crucial to realising the potential of stable isotopic techniques in widely varied fields such as paleoclimatic reconstruction and improvement in crop water use efficiency (Barbour, 2007; Farquhar et al., 2007).

1.2.1 Theories of enrichment

Stable isotope enrichment of leaf water occurs during transpiration and is influenced by factors such as relative humidity, leaf temperature and stomatal conductance.

Stable isotopic enrichment during evaporation from an open water body was first described by Craig and Gordon (1965). Craig & Gordon's evaporative enrichment theory (as seen in Equation 3) states that $H_2{}^{16}O$ evaporates and diffuses more readily than water containing the heavier stable isotopes of deuterium (D) and ^{18}O, thus allowing the heavier isotopes to accumulate at evaporating surfaces of the pool of water (Dongmann et al., 1974; Farquhar et al., 1989; Flanagan et al., 1991). Isotopic fractionation during evaporation and diffusion are described as equilibrium and kinetic isotope effects. The equilibrium effect (with a fractionation factor denoted ε^* and dependent on temperature) results from the phase change from liquid water to water vapour (as seen in Equation 1 and Equation 2). In contrast, the kinetic effect (with a fractionation factor denoted ε_k) is caused by the different diffusion rates of light and heavy isotopes in the water vapour (as seen in Equations 4 and 5) (Barbour, 2007; Bottinga & Craig, 1968; Flanagan et al., 1991).

$$\varepsilon_O^*(‰) = \left[exp\left(\frac{1137}{(273+T)^2} - \frac{0.4156}{273+T} - 2.0667 \times 10^{-3}\right) - 1\right] \times 1000 \qquad \text{Equation 1.}$$

$$\varepsilon_H^*(‰) = \left[exp\left(\frac{24844}{(273+T)^2} - \frac{76.248}{273+T} - 52.612 \times 10^{-3}\right) - 1\right] \times 1000 \qquad \text{Equation 2.}$$

Dongmann et al. (1974), and subsequently Farquhar et al. (1989) and Flanagan et al. (1991) modified the Craig-Gordon model to include the diffusive effects within leaves and formalised this understanding in terms of isotope enrichment above source water (Δ_e) as,

$$\Delta_e = \varepsilon^* + \varepsilon_k + (\Delta_v - \varepsilon_k)e_a/e_i \qquad \text{Equation 3.}$$

where ε_k is dependent on boundary layer and stomatal diffusional conductances (Farquhar, 1998) using Cappa et al. (2003) fractionation effects and 2/3 Poulsen analysis (Kays, 1966);

$$\varepsilon_k^O(‰) = \frac{28r_s + 19r_b}{r_s + r_b} \qquad \text{Equation 4.}$$

$$\varepsilon_k^H(‰) = \frac{25r_s + 17r_b}{r_s + r_b} \qquad \text{Equation 5.}$$

In Equation 3, Δ_e denotes the steady-state enrichment of water at evaporation sites above source water, Δ_v is the isotopic composition of atmospheric water vapour relative to source water, and e_a/e_i is the ratio of ambient to intercellular water vapour pressures. Relative humidity (RH) is a significant factor affecting photosynthesis, gas exchange and water use efficiency (Flanagan et al., 1991). Equation 1 indicates that Δ_e is linearly related to e_a/e_i, and if leaf temperature is equal to air temperature, then e_a/e_i is relative humidity, and leaf water enrichment decreases linearly with RH. However, leaf temperature can depart significantly from air temperature (both higher and lower depending on the leaf energy balance), so the negative linear dependence of Δ_e on RH is a useful approximation only.

Measurements of bulk leaf water isotope enrichment above source water (Δ_L) often reveal that the Craig-Gordon model overestimates the isotopic enrichment in leaf water, suggesting that it is insufficient to fully describe variation in leaf water enrichment.

One modification of the Craig-Gordon model assumes that leaf water is made up of two distinct pools comprising; a) unenriched source water in the xylem and vascular tissue and b) evaporatively enriched water in the mesophyll of leaves (Leaney et al., 1985; Yakir et al., 1994; Yakir et al., 1990).

Others describe enrichment as a “string of pools” or lakes in which leaf water becomes gradually enriched along the water movement pathway. Gan et al. (2002) showed weak evidence supporting the ‘string of lakes’ theory as the spatial patterns of leaf water enrichment was seen to vary with humidity. However, the ‘string of lakes’ theory could not account for the enrichment of bulk leaf water (Gan et al., 2002). The string of pools model is better supported by spatial heterogeneity patterns observed in crop plants such as corn by Wang & Yakir (1995). Helliker and Ehleringer (2000) also show evidence for progressive enrichment in grass species that vary according to interveinal distance and leaf length. The grass species studied exhibited clear differences in enrichment levels of leaf water ^{18}O from base to tip, supporting the need for successively enriched pools (Helliker & Ehleringer, 2000).

Another theory known as the desert river, first introduced by Fontes and Gonfiantini (1967), describes the continual loss of water by evaporation. However, it has been pointed out that the desert river theory may have limited relevance to leaves because it does not consider the contribution of pools at evaporating sites or from the veins as the string of lakes or pools model does (Santrucek et al., 2007). Both the desert river and string of lakes models account for the variation of leaf water enrichment patterns with environmental conditions such as relative humidity, but overestimate average enrichment. Further, none of the models described so far predict the positive relationship observed (in some experiments) between leaf transpiration rate (E) and $1-\Delta_L/\Delta_e$, the fractional difference between measured bulk leaf water and that predicted by the Craig-Gordon model (e.g., Flanagan et al. 1991).

The relationship observed in some experiments between E and $1-\Delta_L/\Delta_e$ prompted the suggestion of an alternative theory by Farquhar et al. (1993) based on the Péclet effect. The Péclet effect theorises that the mesophyll water is less enriched than water at the evaporating sites due to the advection of non-enriched stem water towards the evaporative site, limiting the diffusion of evaporatively enriched water into the leaf (Farquhar et al., 1993).

$$\Delta_L = \frac{\Delta_e\left(1-e^{-\wp}\right)}{\wp} \qquad \text{Equation 6.}$$

The effects of advection and diffusion within the Péclet effect are described by a Péclet number ($\wp$) which depends on velocity and path length, or in simple terms, the ratio of convection to diffusion. $\wp$ determines the strength of the effect based on the velocity of water movement and effective path length for bulk leaf water and source water (see Equation 7) (Barbour et al., 2000).

$$\wp = \frac{EL}{CD} \qquad \text{Equation 7.}$$

where E is in mol $m^{-2}s^{-1}$, L is the effective path length (m), C is the molar density of water (mol m^{-3}), and D is the diffusivity of $H_2{}^{18}O$ in water (m^2 s^{-1}) (Barbour et al., 2021).

Gan et al. (2003) developed the Péclet Effect further by considering both longitudinal and radial Péclet effects, where the longitudinal Péclet number determines enrichment along the leaf via gradients within the veins and the mesophyll tissue (if the mesophyll and veins are hydraulically connected). In contrast, the radial Péclet number determines an average of leaf water enrichment across the whole blade and can be associated with either veinlet water or the leaf lamina (Barbour & Farquhar, 2004). The Péclet effect theory is supported by published observations of positive relationships between E and $1-\Delta_L/\Delta_e$, increasing enrichment in leaf water at leaves’ tips and outer edges (Barbour & Farquhar, 2004; Barbour et al., 2000; Song & Barbour, 2016; Wang & Yakir, 1995).

Following findings from Gan et al. (2002) and Helliker and Ehleringer (2000), Farquhar and Gan (2003) developed a modified Péclet model known as the Farquhar-Gan model. The Farquhar-Gan model considers how longitudinal and radial pathways interact across pools from the xylem, veinlet and lamina, such as through the effects of advection and diffusion to determine the strength of the Péclet effect and the resulting enrichment based on hydraulic connectivity and conductance (Farquhar & Gan, 2003; Holloway-Phillips et al., 2016).

Considering differences in leaf anatomy and potential location of gradients in enrichment may prove helpful for assessing the relevance of a Péclet effect in leaves. Gan et al. (2003) raised an important point by highlighting differences in the Péclet effect with leaf anatomy, such as longitudinal and radial Péclet effects. This idea was supported by Song et al. (2015a), who suggested that steep gradients may occur in vascular tissues, thus demonstrating the relevance of leaf anatomy to Péclet effect enrichment gradients and to leaf water isotopes more generally.

1.2.2 Does leaf hydraulic design influence isotopic enrichment?

A study by Barbour and Farquhar (2004) investigated how the Péclet effect varies with anatomical dimensions and pathways of water movement within leaves. The study examined whether dimensions within the leaf allow the required velocities for isotopic enrichment by quantifying the velocity of water movement within the leaf in relation to slab velocities. The study’s objective was to assess whether scaled effective lengths within wheat leaves allow the development of gradients in the enrichment of $H_2{}^{18}O$ between evaporating sites and veins. Barbour and Farquhar reviewed the pathways of water movement within leaves and highlighted the importance of knowing how water moves through aquaporins to estimate the velocity of its movement and whether a Péclet effect could apply. Another aspect of hydraulic design considered within the study is possible sites of evaporation within the leaf which was explored using modelling techniques (Barbour & Farquhar, 2004).

Barbour and Farquhar found that effective lengths need to be in the order of millimetres for a significant radial Péclet effect to occur and that effective lengths may vary based on pathways of water movements and sites of evaporation. Looking at pathways of water movement, they found that the diffusive process for generating gradients would occur within cell walls and that generation of significant Péclet effects in leaves is possible. Little was known about water movement through aquaporins, although the generation of gradients in enrichment beyond the plasma membrane could be possible if some water moves counter to the net flux (Barbour & Farquhar, 2004).

Through modelling the anatomy of wheat leaves, it was found that significant radial Péclet effects could occur at normal transpiration rates. The different models for sites of evaporation were found to produce vastly different effective lengths for water movement, and a positive relationship was seen between the number of cells travelled and the estimated effective length. A sensitivity analysis also highlighted important assumptions for calculating effective path length as plasmodesmatal frequency and thickness of cell walls and cytosol. Barbour and Farquhar concluded that aspects of leaf hydraulic design, such as anatomy, assumed pathways of water movement, sites of evaporation (affecting velocity), and effective lengths influenced the generation of gradients for enrichment. This conclusion was further supported by evidence for a radial Péclet effect in wheat leaves (Barbour & Farquhar, 2004).

Another recent study by Barbour et al. (2021) investigated whether hydraulic design can explain differences between species in leaf water isotopic enrichment. The hypothesis for the study was "that leaf hydraulic architecture may affect the degree to which gradients in $H_2{}^{18}O$ develop within leaves influencing bulk leaf stable oxygen isotope enrichment and the degree to which the Péclet effect is relevant in leaves". The study aimed to "examine how hydraulic design may be used to explain patterns of leaf water enrichment at the whole leaf level". Twenty-one C_3 species were studied and assigned to one of three hydraulic designs by examining leaf anatomy using a light microscope or published cross sections. The relationship between transpiration rate and the difference between enrichment at sites of evaporation relative to source water and leaf water oxygen isotope composition was also evaluated to test if the hydraulic design can be used to inform the selection of isotopic models.

Results for the selection of isotopic models showed that the Craig-Gordon model is insufficient to explain variation in leaf water oxygen isotope composition and that the two-pool or Péclet effect models are needed to predict variation in the leaf water oxygen isotope composition (Barbour et al., 2021).

Investigation of the three hydraulic designs highlighted differences in apoplastic and symplastic pathways and their relevance to leaf water isotopes. The formation of isotopic gradients was considered more likely in apoplastic pathways than symplastic pathways, as aquaporins and plasmodesmata are too small to support bulk flow. The formation of isotopic gradients in the apoplastic pathway for water movement depends on the ratio of water flow (velocity), effective path length, and $H_2{}^{18}O$ diffusivity. The detection of a Péclet effect depends on the contributions of the different pathways to water transport and variation in hydraulic resistances, which varies with anatomy. Of the three hydraulic designs, only species with Design 2 anatomy showed a clear Péclet effect. This is due to high mesophyll resistance and low expression of aquaporins, resulting in a high velocity for water movement, thus increasing the likelihood of isotopic gradients developing (Barbour et al., 2021). Results from the study combine hydraulic design and isotope theory, suggesting that a Péclet effect is mainly seen in the apoplastic pathway, including bundle sheath extensions; however, it is primarily based on leaf anatomy and requires further testing in a range of environmental conditions (Barbour et al., 2021).

1.2.3 Recommendations and experiments

Across the many studies investigating water transport and isotopic enrichment in plants, various techniques were used to assess certain patterns and identify key influences. The recent development of coupled leaf gas exchange systems with stable isotope laser spectrometry has allowed rigorous testing of leaf water models (Buckley et al., 2017; Cernusak et al., 2018; Farquhar et al., 2021; Loucos et al., 2015; Simonin et al., 2015; Song et al., 2015b). On the other hand, spatially-explicit models of energy and water transport in leaves, combined with sensitivity analysis, have helped to quantify important calculations such as path lengths and contributions of different phases (liquid and vapour) to hydraulic conductance (e.g. Buckley, 2015; Rockwell et al., 2014), (Farquhar et al., 2021; Simonin et al., 2013).

The topics of enrichment and water transport within plants have been of great interest in biology and physiology, with many experiments and theories tested and continually developed over the years; however, there are still aspects which remain unknown. This includes an understanding of variability in water transport pathways and path lengths, and sites of phase changes which are likely to vary between species and according to anatomy. The recent observation that leaf anatomy, specifically leaf hydraulic design, is of particular interest in the recent observation, which influences the observed relationship between E and $1-\Delta_L/\Delta_e$ (Barbour et al., 2021).

The following experiments aim to address the question of the relevance of the Péclet effect to patterns of leaf water isotopic enrichment in monocot and dicot species. Taking the lead from Barbour et al. (2021), natural differences in anatomy and hydraulic design between monocot and dicot leaves will be used to help understand why the Péclet effect may be important in some but not all species. This study will focus on evaporative and progressive enrichment from a basal portion of the leaf towards a more distal region. This study will help to identify differences in the enrichment of leaf water and water transport pathways by investigating differences in leaf hydraulic design, such as the possibility of gradients and variation in different pools. This experiment is designed to test the hypothesis that progressive enrichment from basal to distal regions of leaves is due to the diffusion of enriched mesophyll water into the xylem associated with a localised Péclet effect within the vascular tissue. The findings of this study will be used to help enhance the understanding of leaf water isotope enrichment, which may help to improve the utility of potential applications of leaf water isotope data such as the water use efficiency of crop species and modelling past climates to assist in investigating terrestrial and hydrological fluxes.

Chapter 2

Materials & Methods

2.1 Plant growth conditions

Plant species for the experiments included two monocots; corn (*Zea mays*) and oat (*Avena sativa*), and two dicots; sunflower (*Helianthus annuus*) and foxglove (*Digitalis purpurea*). The plants were grown from seed in trays of seedling mix in greenhouses at the University of Waikato campus from late July to August of 2021. The greenhouses provided an environment with natural light, regulated temperatures, daily watering to field capacity, and checks for pests and diseases. Once the seedlings had germinated, they were thinned out by hand and individually transplanted into 10 L pots filled to 8 L with potting mix for optimal growth. The plants were used for measurements once their leaves were of an appropriate size to fit the gas exchange system, but before reproductive stages were reached. When the plants matured and were large enough to use for measurements, the best eight were selected from each species, and any extra plants were removed. Thrive All-Purpose soluble fertilizer was applied to the slower-growing species such as foxglove as necessary to help encourage growth.

2.2 Coupled leaf gas exchange and isoflux measurement

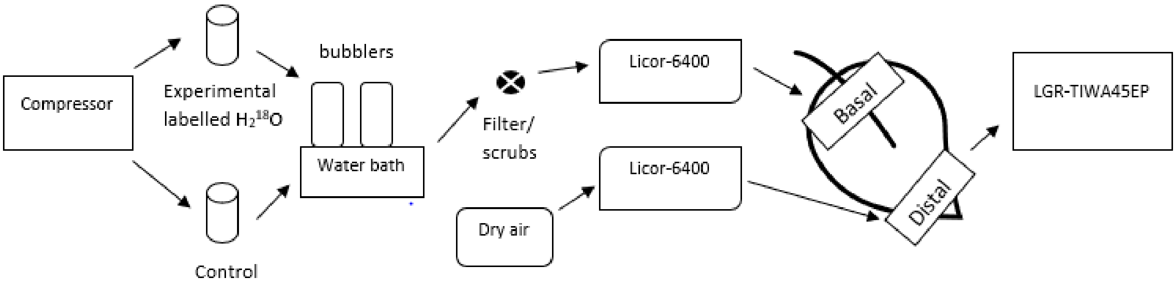

Figure 2.1 Experimental design for gas exchange and stable isotope measurements.

^{18}O-labelled water was prepared using tap water mixed with 10 atom % $H_2{}^{18}O$ to produce 152‰, which was used to fill one of the two bubblers. The labelled water was only enriched in ^{18}O and not in D. The second bubbler was filled with tap water to form a control.

Gas exchange measurements of leaf transpiration and isofluxes were recorded at 1-minute intervals across two LiCor portable photosynthesis systems (Li6400xt, LiCor Inc.) fitted with 2×3 cm chambers with a red-blue LED irradiance source (Li6400-18A). Both chambers had controlled settings with a CO_2 inlet concentration of 400 µmol mol^{-1}, a flow rate of 500 mol s^{-1}, a light level of 1500 µmol m^{-2} s^{-1} and a leaf temperature of 25 °C. The inlet water vapour concentration for the distal chamber was completely dried using the LiCor 6400 drierite trap to ensure the water vapour concentration output (connected to the laser for isotope measurements) was only from leaf transpiration (see Figure 2.1).

The rates of transpiration for the experiment to determine the relationship between E and $1-\Delta_L/\Delta_e$ (Experiment Three) were varied by manipulating flow rate, relative humidity, CO_2 concentration and light levels (Barbour et al., 2021). In this case, the 2×6 cm narrow leaf chamber (6400-11) was used with the red-green-blue light source (6400-18 Å) set to white colour.

2.3 Stable isotope measurement and calibration

2.3.1 Water vapour isotopes

Measurements for gas exchange and stable isotopes were done by species (six to eight leaves measured per species), and each leaf was selected based on health and size. Stable isotope measurements were taken by connecting the distal chamber from the LiCor system to a Los Gatos Research (LGR) water vapour gas analyser (TIWA-45EP) (Los Gatos Inc., Mount View, CA). The LGR and LiCor systems were calibrated following the protocol described by Simonin et al. (2013) between periods of measurements for each species. The calibration included completing a multiple linear regression and correction of raw isotope data for any concentration dependence.

2.3.2 Liquid water $\delta^{18}O$ by H_2O-CO_2 equilibration

Water sample standards and leaf water samples were analysed using an H_2O-CO_2 equilibration method similar to that described in Song and Barbour (2016), except that an LGR-CO_2 isotope laser (CCIA2-912, Los Gatos Inc., Mount View, CA) was used for the measurements. Three different water standards were used: -25.9‰, -0.8‰, and 57.2‰ ^{18}O mL^{-1} and five samples were prepared of each standard. The water standards were prepared for analysis by injecting 200 µL into 100 mL glass vials flushed with 2% CO_2, then sealed with a polytetrafluoroethylene (PTFE) lined butyl septa crimp cap. The leaf samples were prepared in a separate set of 100 mL glass vials by sealing the leaf tissue into the vial after flushing with 2% CO_2. The water standards and leaf water samples were then allowed to equilibrate isotopically for 48 hours at room temperature before analysis.

Determining the leaf area required to produce the ratio of water to CO_2 in the glass vials was done as described below (Song & Barbour, 2016). Leaf water samples were taken from live leaves attached to plants with a leaf area of either 13.2 cm^2 (Experiment Two) or 12 cm^2. Five tissue samples were cut from the tip and base of foxglove leaves using a template of 13.12 cm^2 and weighed individually for fresh leaves. The tissue samples were then sealed in paper envelopes and dried at 60 °C for 48 hours before weighing again. The amount of water was calculated by the weight of the fresh leaf sample subtracted from the dried sample, then converted from g to μL and divided by the leaf area.

2.4 Experimental design

2.4.1 Experiment One: Carry forward of labelled vapour from basal to distal leaf portions in four species

Experiment One aimed to test for evidence of a Péclet effect by examining the potential for carry-forward of leaf water enrichment from basal to distal leaf portions across monocot and dicot leaves.

The leaf was equilibrated within the chambers for 2 to 3 hours before measurements using the unlabelled water vapour as the inlet to the basal chamber. After stomatal conductance, the transpiration rate and $\delta^{18}O$ of transpired vapour from the distal leaf portion had stabilised, the bubbler and the soda lime trap were switched to the labelled water. When switching from unlabelled to labelled water vapour, the soda lime CO_2 was changed to avoid memory effects. Measurements were then run for 3 to 4 hours per leaf until the isotope composition of transpired vapour from the distal chamber had stabilised once more.

After gas exchange and stable isotope measurements, the chamber placement was labelled on the leaf and photographed to calculate the relative distance along the leaf.

Measurements took place over two months, from September to October 2021, except for foxglove, which was measured in January 2022. This included six to eight leaves per species (3-4 control and 3-4 label).

Concurrent measurements were also made of transpired vapour deuterium composition, but as no deuterium label was added to the bubbler water, these measurements acted as an internal control for the ^{18}O labelling experiment.

2.4.2 Experiment Two: Spatial variation in Foxglove

Experiment Two aimed to quantify spatial variation in $\delta^{18}O$ of bulk leaf water for foxglove. Previous studies have determined that monocots, including corn, have strong patterns of enrichment from the basal to the distal portion of leaves (Helliker & Ehleringer, 2000). Furthermore, previous work demonstrated that spatial variation of $\delta^{18}O$ in sunflower leaves was present but showed relatively small increases in enrichment from the base to the tip between 0‰ and 3.3‰ (Wang & Yakir, 1995).

Five samples of each standard were prepared using the same methods described earlier to correct values after analysis. Leaf tissue samples were prepared from four foxglove plants, using one leaf from each and taking five samples along either side of the main vein using a template for the leaf area (13.12 cm^2). During the 48-hour equilibration period, the leaf tissue samples were stored in boxes to ensure darkness and minimise respiration. After sampling, the leaf was removed from the plant and photographed for future reference.

Once the equilibration period had concluded, the air was extracted from the samples individually using an air-tight syringe with a valve (~60 mL) and injected into an air-tight 5 L Tedlar bag to which 3-4 L of pure nitrogen gas was added before analysis using the LGR-CO_2 isotope laser. Values were recorded for measurement start and end time, CO_2 concentration and $\delta^{18}O$ for each sample during analysis.

2.4.3 Experiment Three: Craig Gordon and Péclet effect relevance in Foxglove

Experiment Three aimed to determine the relevance of the Péclet effect in foxglove by comparing $H_2{}^{18}O$ enrichment in bulk leaf water with the Craig-Gordon model predicted enrichment, following Song et al. (2015a) and Barbour et al. (2021). Previous work by Barbour et al. (2021) determined that the Péclet effect was relevant for monocots, including corn and wheat but was not relevant in sunflower. Measurements were made using four foxglove plants with three to five leaves from each plant. Stomatal conductance and transpiration rates were measured using the LiCor system with a leaf chamber area of 12 cm^2. The selected leaf was placed in the chamber, and levels of CO_2, light and relative humidity were manipulated to help control stomatal conductance and adjust rates of transpiration; measurements began after stomatal conductance had stabilised. The leaf chamber had dry air entering and was set to control leaf temperature at 25 °C. Other settings of the leaf chamber were manipulated, including flow rate between 300 to 600 mol s^{-1}, the inlet CO_2 concentration of 100 to 1000 µmol mol^{-1} and light levels between 50 to 1300 µmol m^{-2} s^{-1} using an RGB light source (6400-18 Å) set to white. During the measurement period, records of the start time, stomatal conductance and transpiration rates were made for each leaf to achieve a range of different transpiration rates across the measurements for comparison. After finishing measurements of stomatal conductance and transpiration rates, the area of the chamber was outlined before removing the leaf from the chamber. Immediately following the removal of the leaf from the chamber, a tissue sample was taken from within the outlined area using a template and placed in a 100 mL glass vial which was then flushed with 2% CO_2 and sealed. This process was repeated for each leaf measured, and after completion of measurements, the leaf tissue samples were given 48 hours to equilibrate before measurement using the LGR-CO_2 isotope laser, as described earlier.

2.5 Data analysis

After measurements were obtained for each species using multiple individual plants in Experiment One, the change in isotopic composition over time was assessed, including calculated parameters for transpiration rates, vapour pressure deficit (VPD), conductance and leaf temperature. The data was imported from LiCor systems and the LGR after each day’s measurements for later processing using Excel. One-minute averages were calculated for values of $\delta^{18}O$ and δD from the LGR data. The averaged values for $\delta^{18}O$ and δD were then corrected using an equation from the calibration file for the time in which the measurements took place. The data from both LiCor systems and the LGR was then matched and aligned to account for time differences between the equipment.

The resulting time series data are presented in graphs. Average and standard errors for the change in isotope compositions between the start and the end of the labelling period were calculated for control and experimental leaves for each of the four species.

Data from Experiment Two was processed in Excel by taking 1-minute averages for δ^{18}O of CO_2 equilibrated with leaf water (corrected against water standards using a multiple regression) and ANOVA to assess spatial variation in foxglove. The resulting values were then used to plot δ^{18}O on diagrams for each leaf based on the position of the leaf sample taken and analysed for patterns of enrichment.

The data from Experiment Three was used to calculate the change in transpiration rates, and the fractional difference between bulk leaf water and Craig-Gordon predicted δ^{18}O $(1-\Delta_L/\Delta_e)$ after aligning times and correcting values using the equation from the calibration file. The data was processed in Excel, and the calculations were similar to those for Experiment One. The calculated values were then plotted, and the relationship was statistically tested using a least squares linear regression.

Chapter 3

Results

3.1 Experiment One

3.1.1 Corn

Among the corn leaves, the stomatal conductance was generally found to be slightly lower in the basal chamber in contrast to the distal chamber for both the labelled and control leaves, although the difference was usually small, 18% (Figure 3.1A and 3.3A; Appendix). Across all observations, the measured stomatal conductance and transpiration rates showed a general decreasing trend over time for both control and labelled leaves. Note that the transpiration rates were lower in the basal chambers compared to the distal chambers for all corn leaves due to the higher vapour pressure of the airstreams entering the basal chambers (i.e., water vapour was removed from the inlet air streams for the distal chambers).

Both labelled and control leaves showed a rapid increase and then approached a constant $\delta^{18}O$ of transpired vapour between -16.6‰ and 12.0‰ during the stabilisation phase with tap water in the bubbler. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber showed a significant increase in $\delta^{18}O$ over time, reaching between 11.3‰ and 14.7‰, indicating that the $H_2{}^{18}O$ vapour label was transported within the leaf from the basal towards more distal locations (Figure 3.1C; Appendix). In contrast, the control leaves showed a decreasing trend of $\delta^{18}O$ over time, showing no evidence of enrichment despite fluctuations (Figure 3.3C; Appendix).

Both labelled and control leaves showed a rapid increase and then approached a constant δD of transpired vapour between -49.9‰ and 23.7‰ during the stabilisation phase with tap water in the bubbler. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber showed a constant δD, with no significant changes over time (Figure 3.1E; Appendix). The control leaves showed a slight gradual decreasing trend over time, despite being variable due to fluctuations (Figure 3.3E; Appendix).

The leaf water oxygen isotope composition calculated from the transpired water vapour composition for the leaf portion inside the distal chamber when the basal leaf chamber was exposed to the vapour label showed a gradual linear increase in δ^{18}O over time after the label was applied, reaching between 46.1‰ and 50.2‰, and again suggesting a carry-forward of the labelled water along the leaf (Figure 3.2D; Appendix). The majority of the control group (except Corn plant 7) showed a constant leaf water δ^{18}O with a minor decrease over time (Figure 3.4D; Appendix).

The calculated leaf water hydrogen isotope composition for the ^{18}O-labelled leaves was relatively constant over time (Figure 3.2F; Appendix). The δD of leaf water for the majority of the control leaves (except Corn plant 7) was relatively constant, with a minor increase seen over time (Figure 3.4F; Appendix).

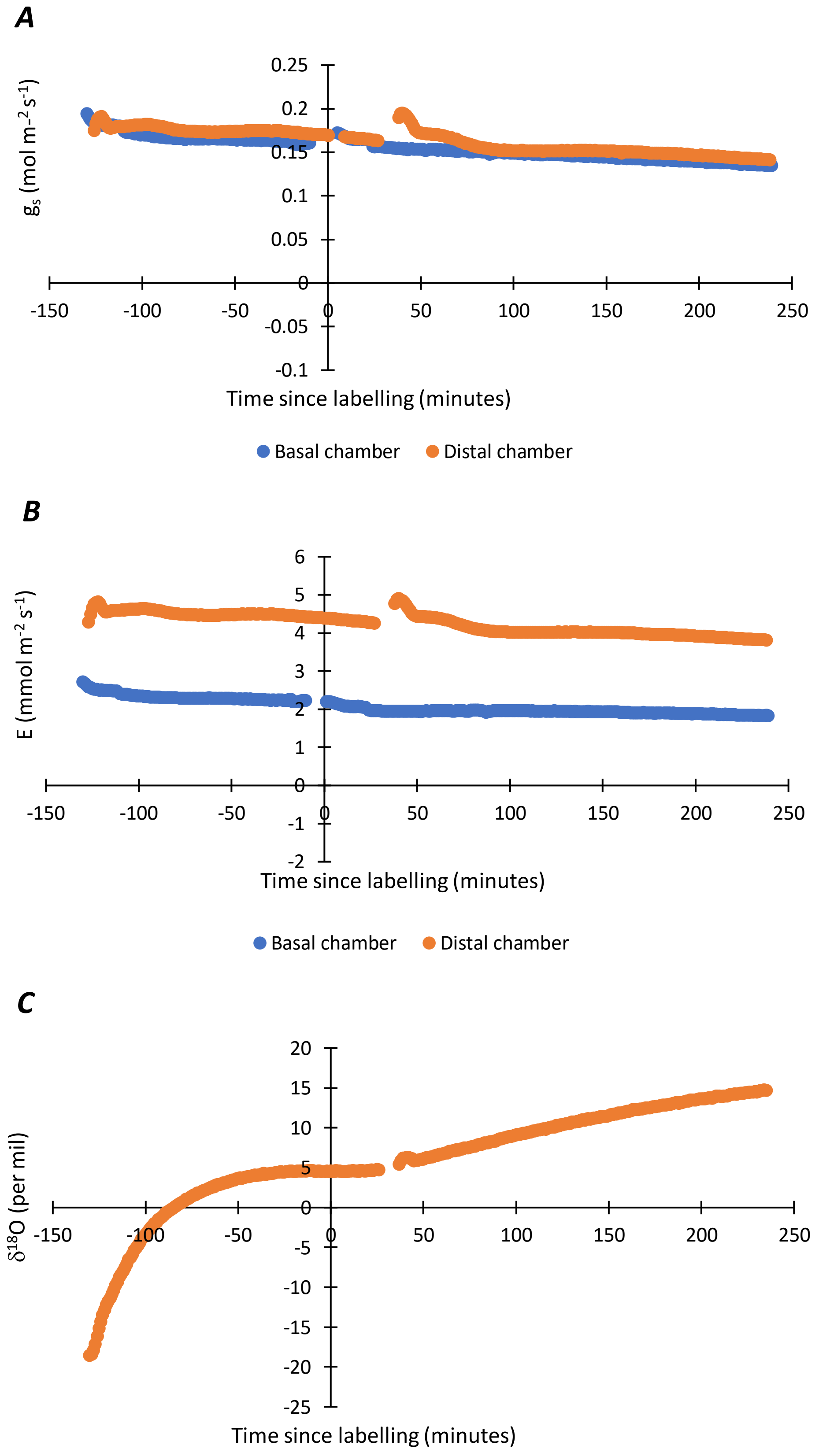

Figure 3.1 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 2 (label).

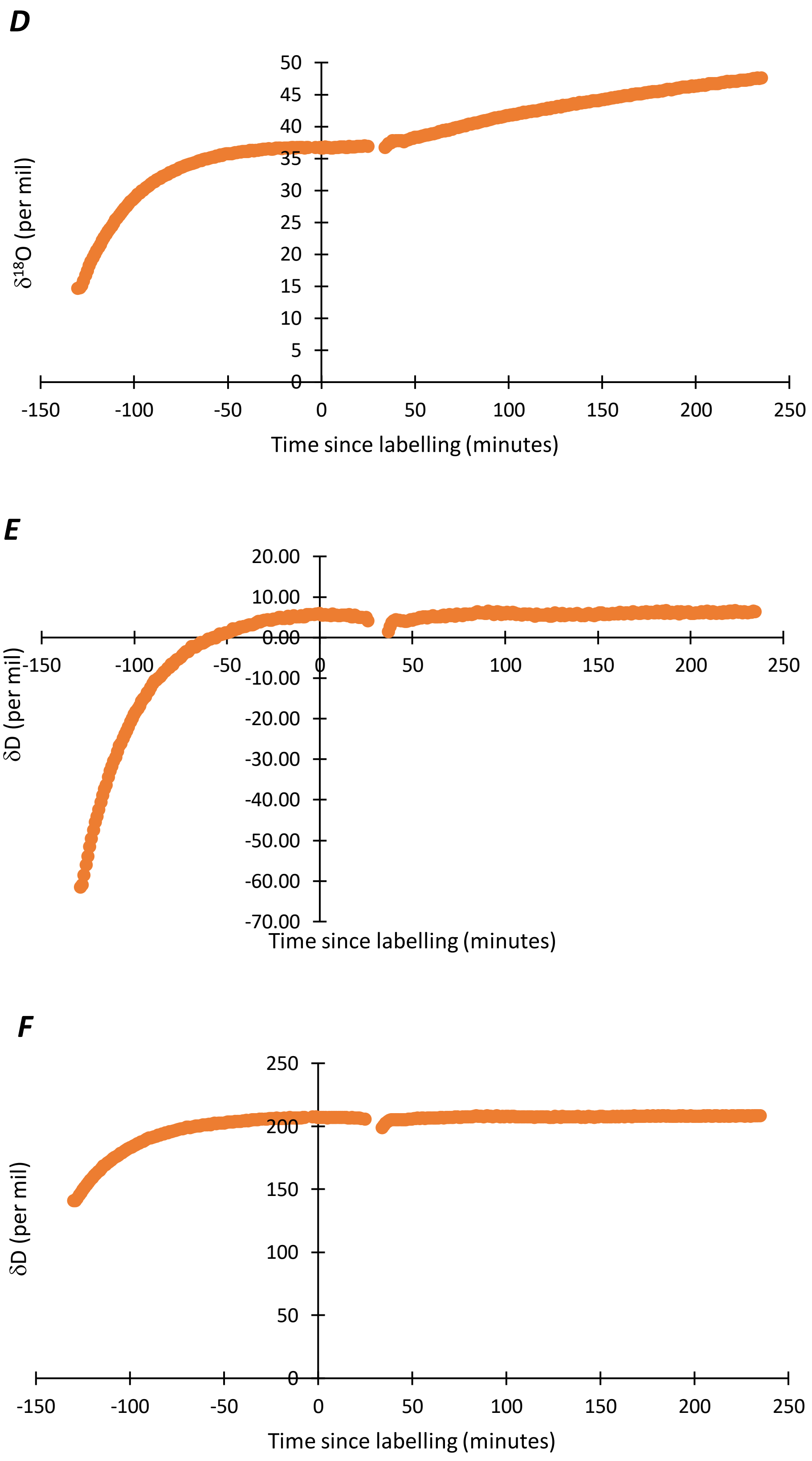

Figure 3.2 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 2 (label).

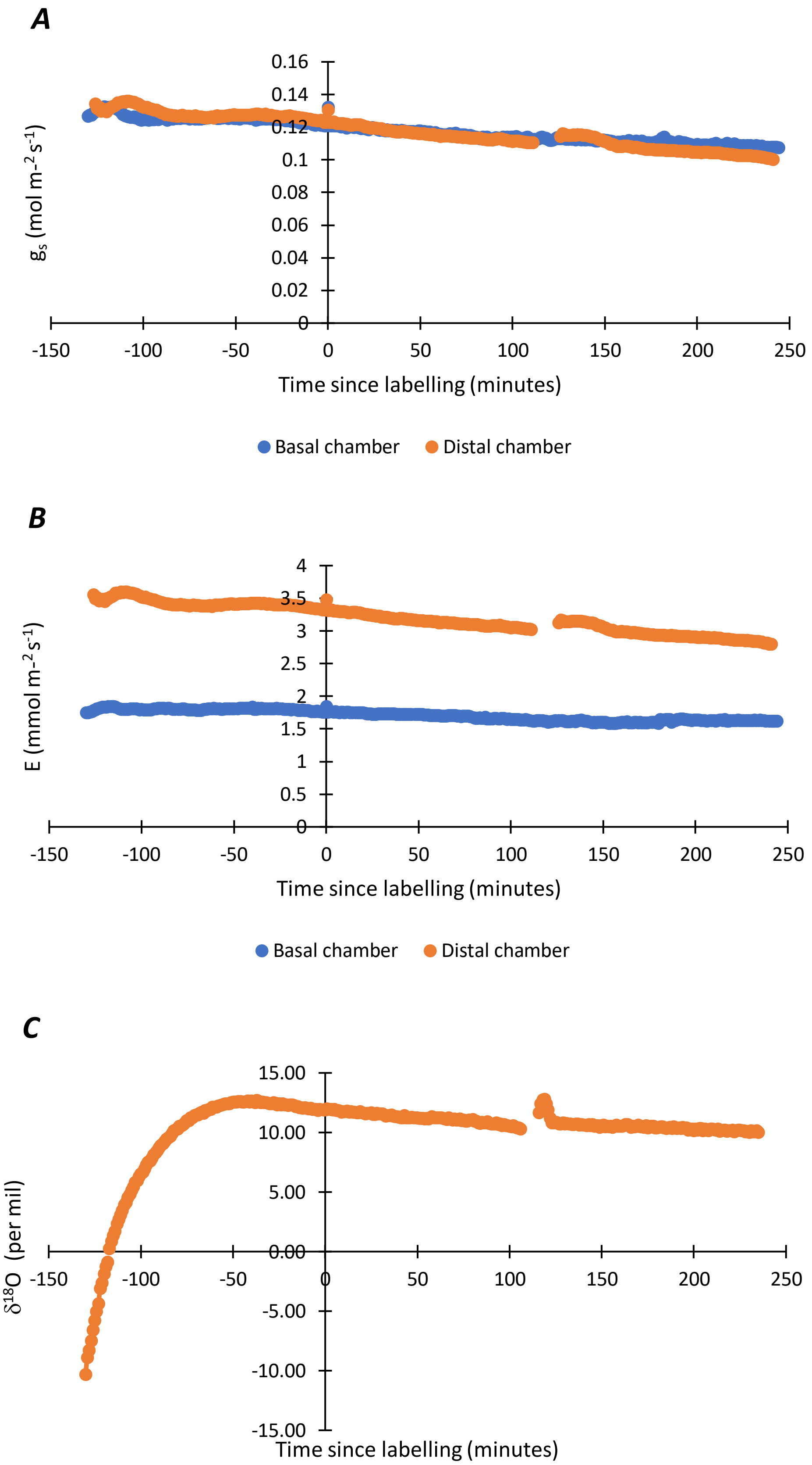

Figure 3.3 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 5 (control).

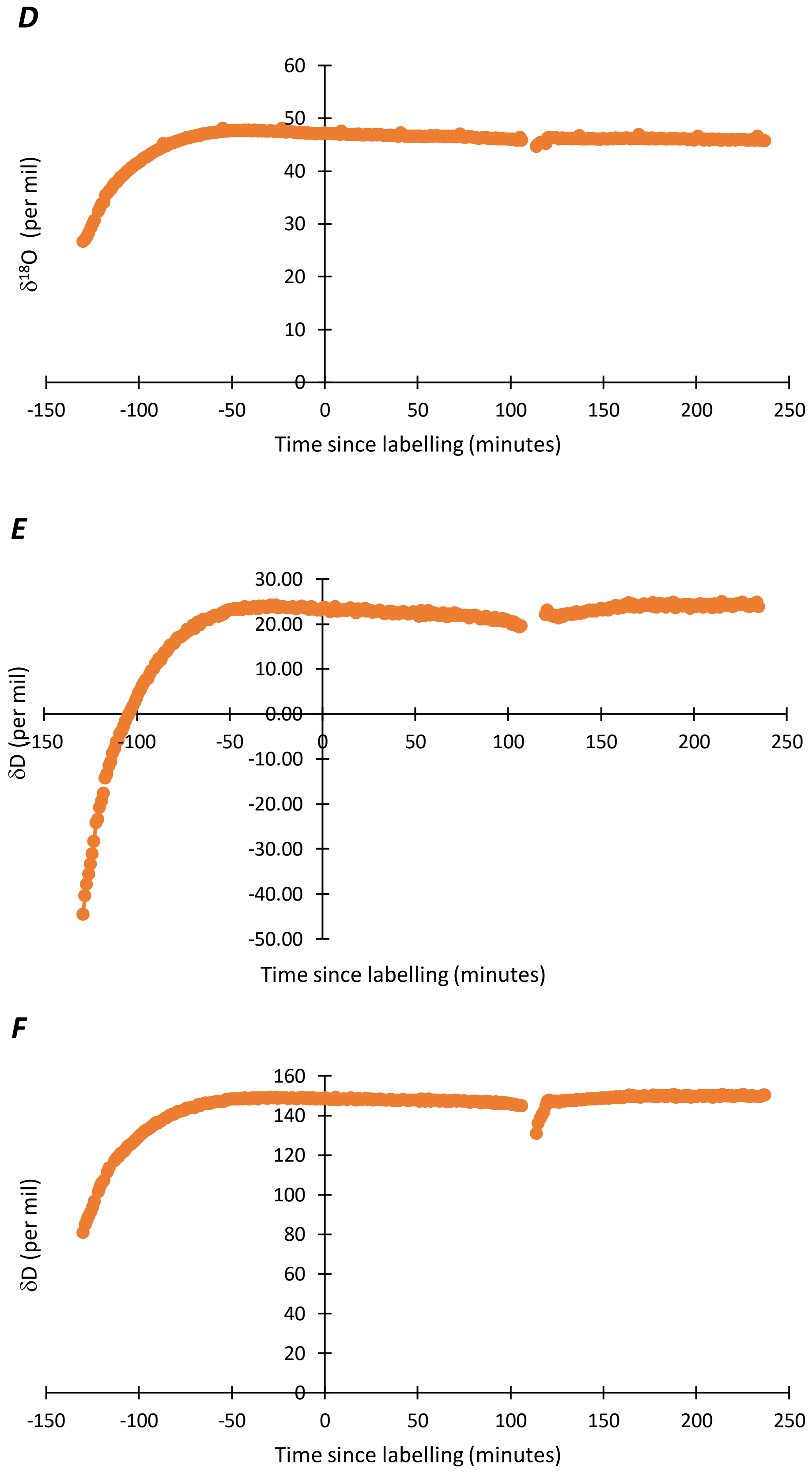

Figure 3.4 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 5 (control).

3.1.1.1 Comparison of changes in isotope composition between labelled and control leaves

The labelled leaves had a significant change in δ^{18}O for both transpired vapour and calculated leaf water between the start and end values ($P = 0.003^{**}$; $P = 0.004^{**}$) while the control leaves showed no significant change in δ^{18}O. The difference in δ^{18}O for the labelled leaves was positive, indicating the presence of the label in the distal part of the leaf. In contrast, the control leaves did not show evidence of changes in δ^{18}O and, generally, showed a slight decrease over time (except for Corn plant 7). The change in δD for transpired vapour and leaf water in both the labelled and control leaves was variable, with both small increases and decreases over time, none of which were statistically significant (Table 3.1). This is consistent with the natural abundance of δD present in both the control and labelled vapour and indicates that changes in δ^{18}O of transpiration from the distal chamber for labelled leaves were due to the label rather than any changes in leaf environmental conditions.

Table 3.1 Changes in transpired vapour and leaf water isotope composition (‰) for Corn.

Group	**Plant number**	**Label (Corn)**				**Plant number**	**Control (Corn)**			
		$\delta^{18}O_{trans}$	δD_{trans}	$\delta^{18}Oe$	δDe		$\delta^{18}O_{trans}$	δD_{trans}	$\delta^{18}Oe$	δDe
Start	**1**	3.2	3.4	37.4	127.5	**5**	12.0	23.6	47.2	148.6
	2	4.6	5.5	38.7	129.5	**6**	4.4	3.4	38.2	127.1
	8	5.3	4.3	39.7	133.2	**7**	3.5	0.8	36.9	124.1
End	**1**	10.8	1.2	45.6	126.7	**5**	10.3	24.2	46.1	149.8
	2	13.8	6.1	48.5	130.6	**6**	4.4	1.8	38.6	126.7
	8	12.7	7.1	47.5	131.8	**7**	5.5	5.8	39.8	130.7
Difference	**1**	7.6	-2.2	8.2	-0.8	**5**	-1.7	0.6	-1.1	1.2
	2	9.1	0.7	9.8	1.1	**6**	-0.1	-1.6	0.5	-0.4
	8	7.4	2.8	7.8	-1.5	**7**	2.0	5.0	2.9	6.6
Average of differences		8.0 ± 0.6	0.4 ± 1.4	8.6 ± 0.6	-0.4 ± 0.8	**-**	0.1 ± 1.1	1.3 ± 1.9	0.8 ± 1.2	2.5 ± 2.1
***P*-value**		0.003	0.727	0.004	0.267	**-**	0.003	0.727	0.004	0.267

3.1.2 Foxglove

Among the foxglove leaves, the stomatal conductance was seen to be lower in the distal chambers compared to the basal chambers for the majority of both the labelled and control leaves, with a 5% difference (Figure 3.5A and Figure 3.7A; Appendix). Transpiration rates were lower in the basal chambers compared to the distal chambers for most of the labelled and control leaves, with a 19% difference (Figure 3.5B and Figure 3.7B; Appendix). The measured stomatal conductance and transpiration rates showed a general decreasing trend over time for the majority of both control and labelled leaves. However, a few exceptions were seen, such as higher rates of transpiration in the distal chambers for Foxglove leaves 3 and 4 and lower rates of stomatal conductance in the basal cambers for Foxglove leaf 5. Additionally, Foxglove leaves 2 and 6 showed a contrasting trend as stomatal conductance and transpiration rates increased rather than decreased (Appendix).

Both labelled and control leaves showed a gradual linear increase before approaching a constant δ^{18}O of transpired vapour between -17.5‰ and 0.5‰ during the stabilisation phase with tap water in the bubbler. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber showed a small gradual increase in δ^{18}O over time, reaching between -11.9‰ and -8.5‰ (Figure 3.5C; Appendix). The observed increase in transpired vapour of δ^{18}O indicates that the $H_2^{18}O$ vapour label was transported within the leaf from the basal regions towards the distal regions. In contrast, the control leaves showed a smaller increase in δ^{18}O over time, reaching -10.7‰ compared to the leaves exposed to the ^{18}O-label, presenting no evidence of enrichment (Figure 3.7C; Appendix).

Both labelled and control leaves showed a rapid linear increase before approaching a constant δD of transpired vapour between -45.0‰ and -4.5‰ during the stabilisation phase with tap water in the bubbler. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber showed an increase in δD over time, reaching between -19.1‰ and -4.5‰, suggesting a change in environmental conditions (Figure 3.6E; Appendix). Whereas the control leaves showed a smaller increase in transpired vapour δD over time compared to the leaves exposed to the ^{18}O-label (Figure 3.8E; Appendix).

The leaf water oxygen isotope composition calculated from the transpired water vapour composition for the leaf portion inside the distal chamber when the basal leaf chamber was exposed to the vapour label showed a gradual linear increase in $\delta^{18}O$ over time after the label was applied, reaching between 26.4‰ and 29.8‰. The observed increase in leaf water $\delta^{18}O$ again indicates a carry-forward of the labelled water along the leaf (Figure 3.6D; Appendix). A smaller increase in leaf water $\delta^{18}O$ was seen in the control leaves compared to the leaves exposed to the ^{18}O-label (Figure 3.8D; Appendix).

The calculated leaf water hydrogen isotope composition for the ^{18}O-labelled leaves showed a gradual increase of δD over time, reaching between 107.5‰ and 120.2‰, suggesting a change in environmental conditions within the distal chamber and consistent with the changes in stomatal conductance and transpiration rate (Figure 3.6F). The control leaves showed a smaller increase of δD for leaf water over time compared to the ^{18}O-labelled leaves (Figure 3.8F).

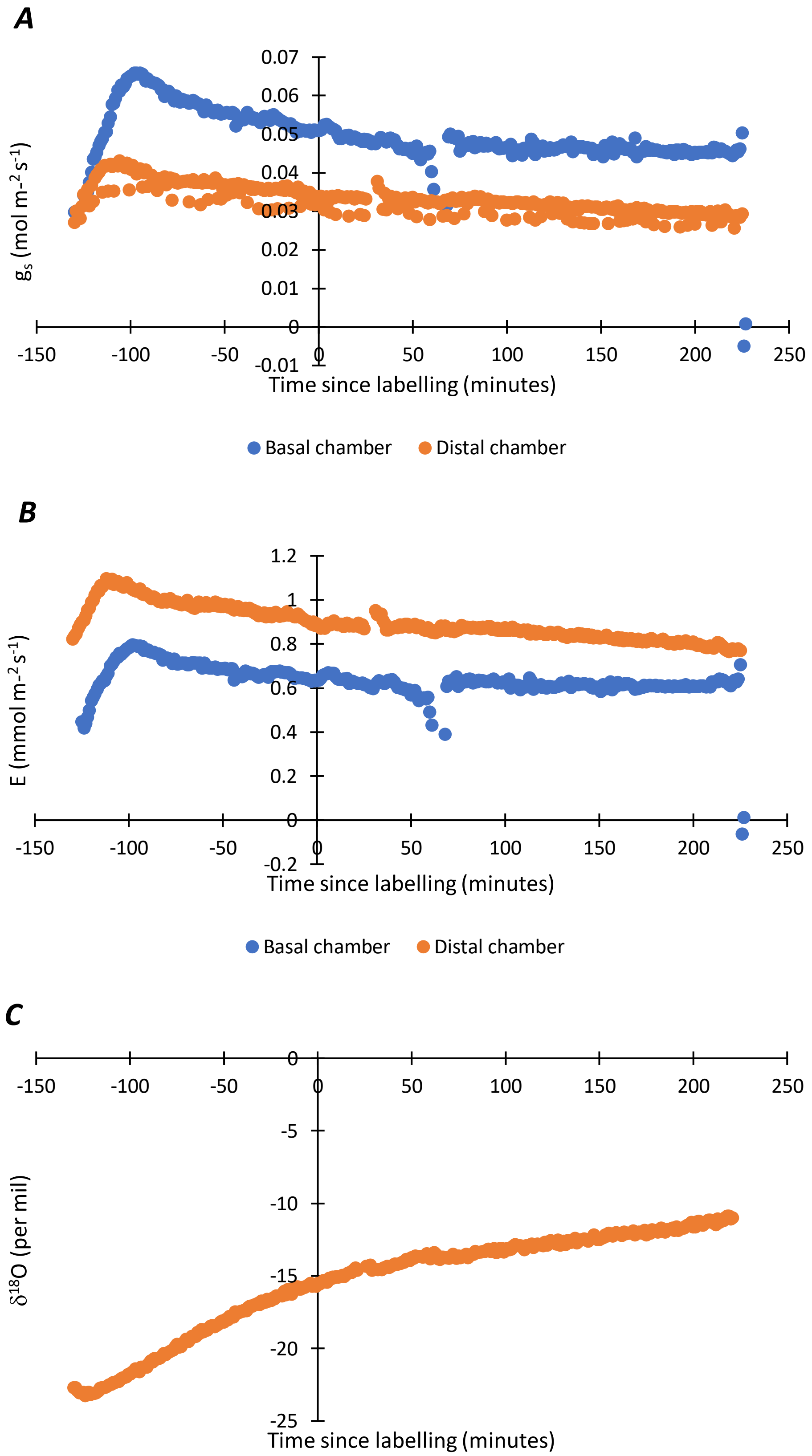

Figure 3.5 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Foxglove plant 1 (label).

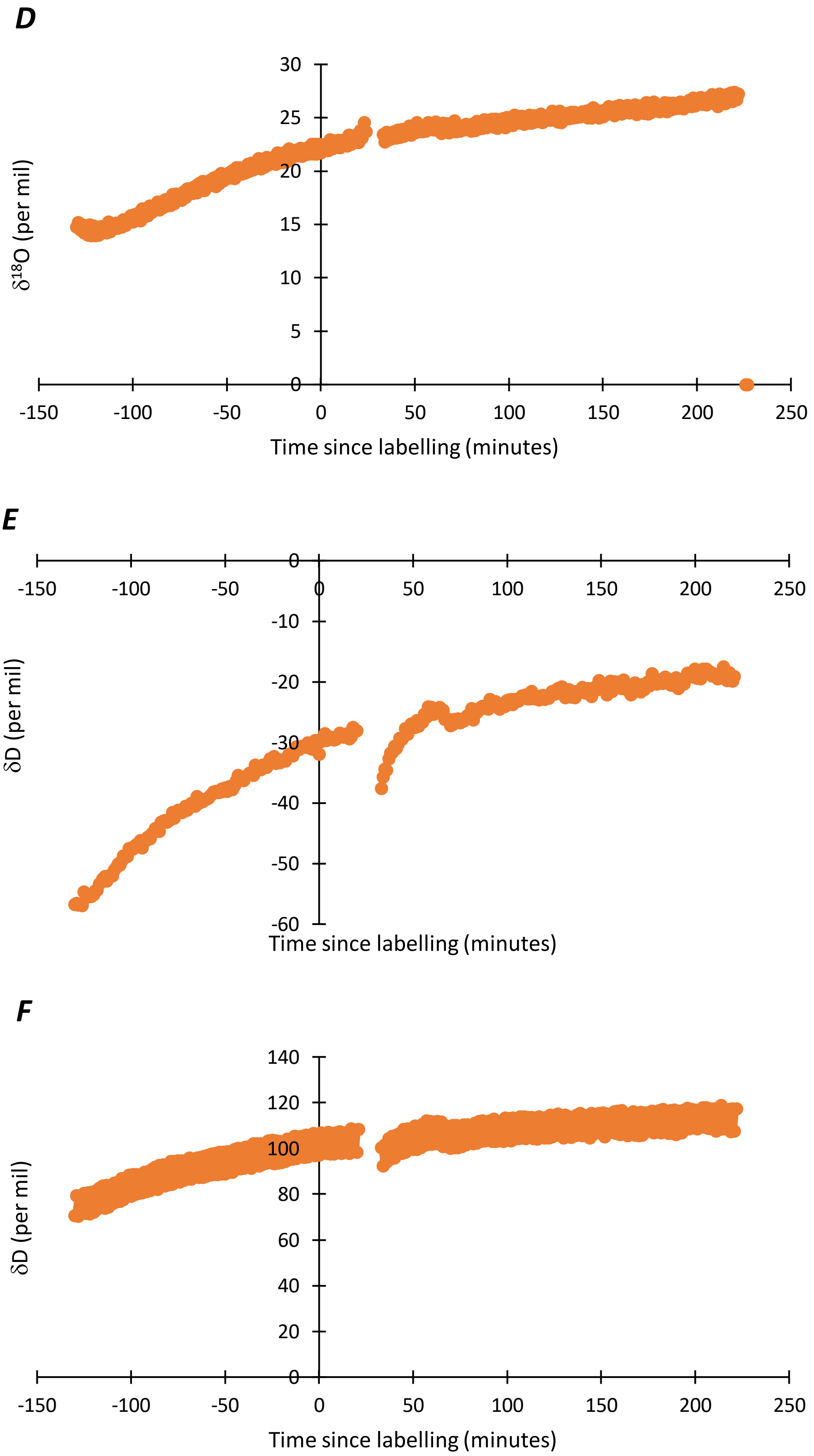

Figure 3.6 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Foxglove plant 1 (label).

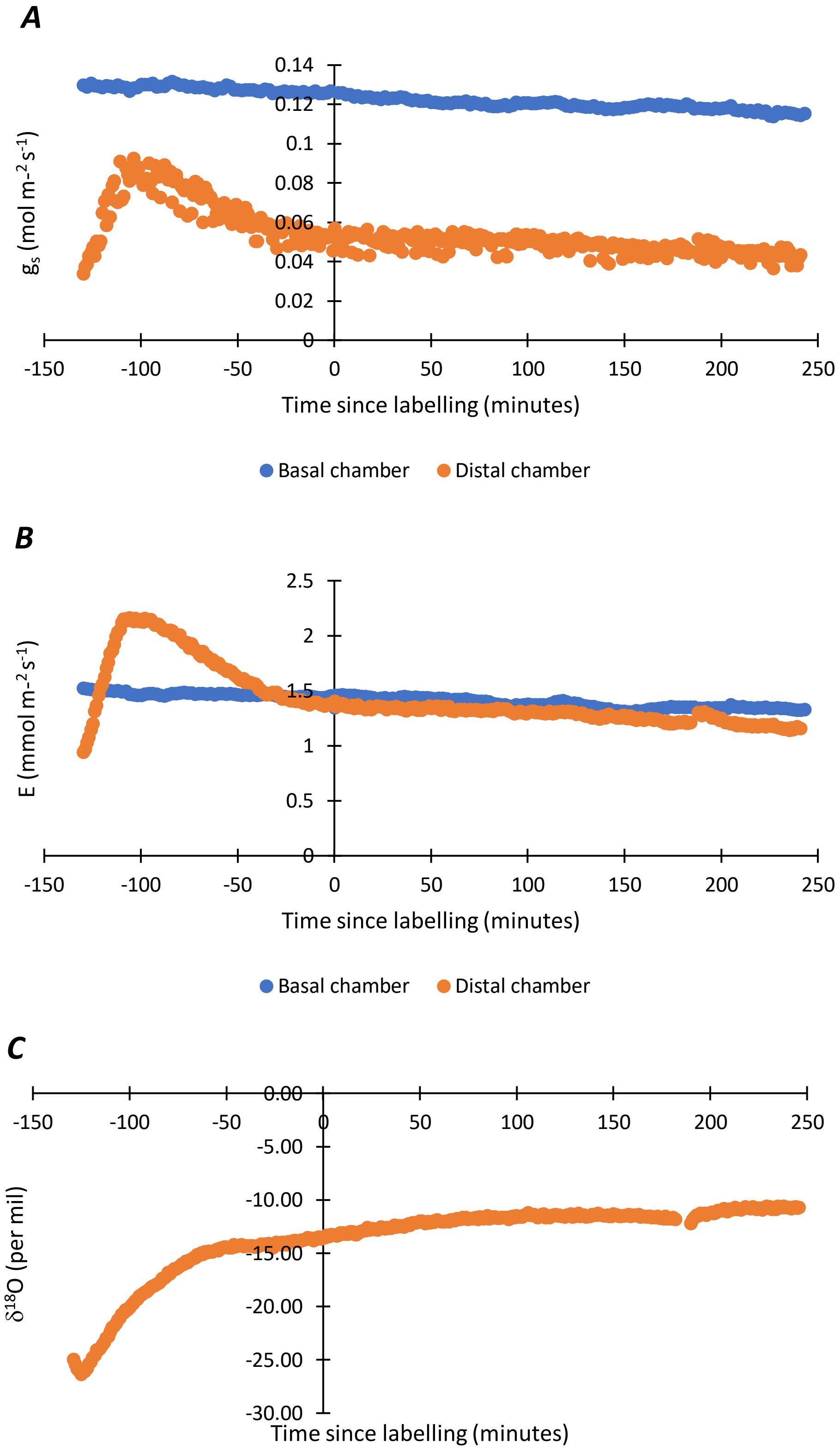

Figure 3.7 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Foxglove plant 4 (control).

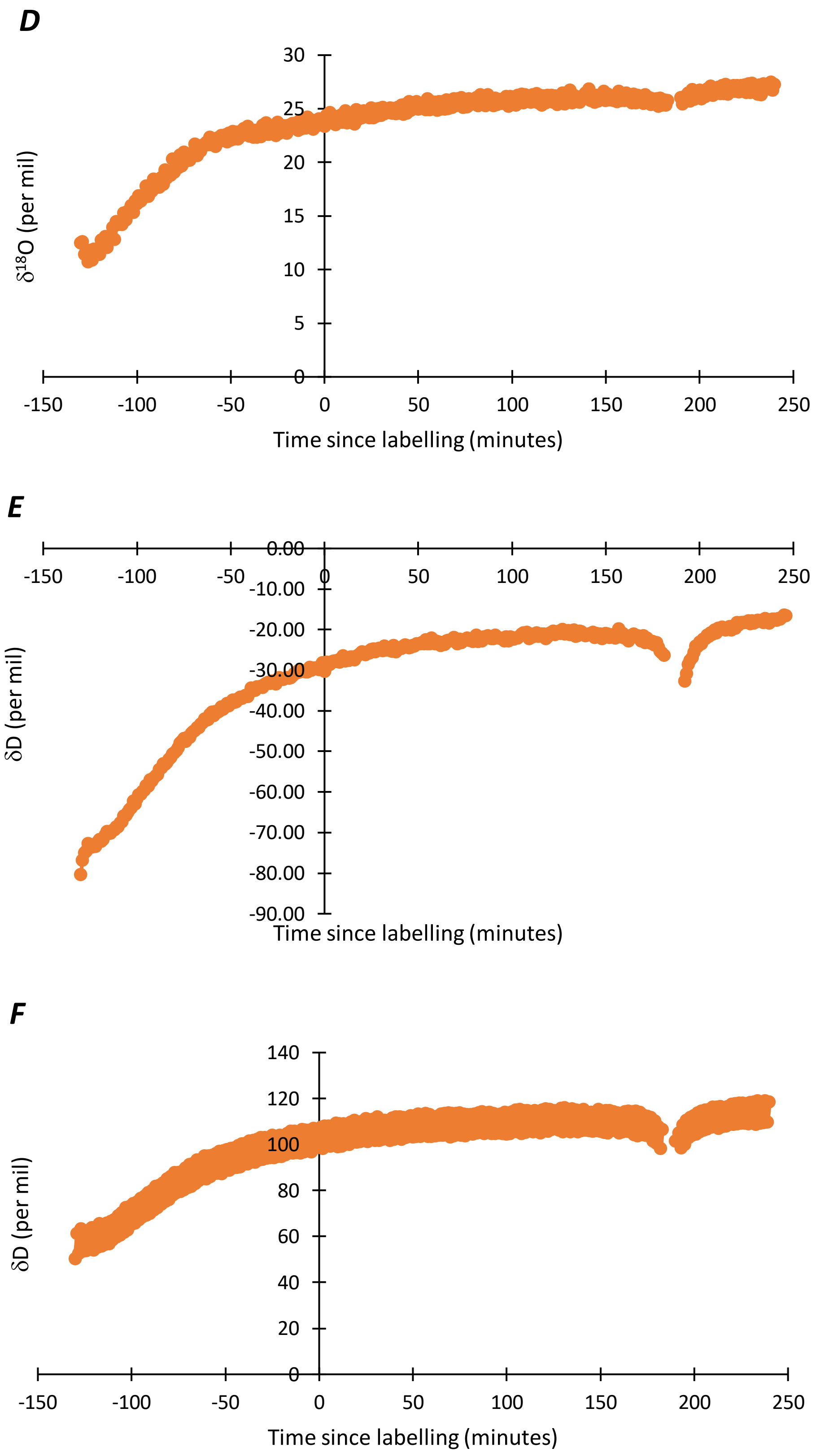

Figure 3.8 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Foxglove plant 4 (control).

3.1.2.1 Comparison of changes in isotope composition in labelled and control leaves

Both the labelled and control leaves had a significant change in δ^{18}O for both transpired vapour and calculated leaf water between the start and end values ($P = 0.011^{**}$; $P = 0.022^{*}$), although smaller increases were observed in the latter. The δD for transpired vapour and leaf water in both the labelled and control leaves were seen to increase over time, with greater increases seen in the former; however, none of the changes was found to be statistically significant (Table 3.2).

Table 3.2 Changes in transpired vapour and leaf water isotope composition (‰) for Foxglove.

	Plant number	**Label (Foxglove)**				**Plant number**	**Control (Foxglove)**			
		$\delta^{18}O_{trans}$	**δD_{trans}**	**$\delta^{18}Oe$**	**δDe**		**$\delta^{18}O_{trans}$**	**δD_{trans}**	**$\delta^{18}Oe$**	**δDe**
Start	**1**	-15.7	-30.6	19.8	175.0	**2**	-1.0	-5.6	33.0	199.2
	3	-10.6	-11.5	25.0	194.2	**4**	-13.7	-29.7	21.5	175.8
	5	-18.8	-34.4	17.6	170.9	**6**	-3.9	-11.6	30.1	192.6
End	**1**	-11.3	-18.6	24.4	187.1	**2**	1.3	2.3	35.9	207.0
	3	-3.6	0.8	32.4	206.5	**4**	-11.8	-22.5	23.5	183.1
	5	-13.5	-8.6	22.8	197.4	**6**	-2.9	-6.2	30.7	198.4
Difference	**1**	4.4	11.9	4.6	12.1	**2**	2.3	7.9	2.9	7.9
	3	7.0	12.2	7.4	12.3	**4**	1.9	7.2	2.0	7.4
	5	5.3	25.8	5.2	26.6	**6**	1.0	5.4	0.5	5.8
Average of difference		5.6 ± 0.8	16.7 ± 4.6	5.8 ± 0.8	17.0 ± 4.8	**-**	1.7 ± 0.4	6.9 ± 0.8	1.8 ± 0.7	7.0 ± 0.6
***P*-value**		0.011	0.102	0.022	0.109	**-**	0.011	0.102	0.022	0.109

3.1.3 Oat

Among the oat leaves, stomatal conductance was generally found to be lower in the distal chamber compared to the basal chamber, with 11% difference for both the labelled and control leaves (Figure 3.9A and Figure 3.11A; Appendix). Transpiration rates were generally found to be lower in the basal chambers compared to the distal chamber for both the labelled and control leaves, with a small difference of 26% due to the higher vapour pressure of the airstreams entering the basal chambers (i.e., water vapour was removed from the inlet air streams for the distal chambers) (Figure 3.9B and Figure 3.11B; Appendix). Across all observations for measured stomatal conductance and transpiration rates, a general decreasing trend was seen over time, with the basal chamber displaying more frequent minor fluctuations compared to the distal chamber for the majority of the labelled and control leaves. However, Oat leaves 3 and 4 showed a small increasing trend in the basal chamber for both stomatal conductance and transpiration rates (Appendix).

Most of the labelled and control leaves showed a rapid increase before approaching a constant $\delta^{18}O$ of transpired vapour between -0.9‰ and 11.6‰ during the stabilisation phase with tap water in the bubbler, although Oat leaves 4 and 6 showed a gradual rather than a rapid increase in $\delta^{18}O$ during the stabilisation phase. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber showed a significant increase in $\delta^{18}O$ over time, reaching between 10.7‰ and 20.4‰, indicating that the $H_2{}^{18}O$ vapour label was transported within the leaf from the basal regions towards the distal regions at least in two of the three labelled leaves (Figure 3.9C; Appendix). In contrast, the control leaves showed a gradual decrease in $\delta^{18}O$ over time, presenting no evidence of enrichment despite fluctuations (Figure 3.11C; Appendix).

Most of the labelled and control leaves showed a rapid increase before approaching a constant δD of transpired vapour between -43.1‰ and 46.0‰ during the stabilisation phase with tap water in the bubbler. An exception to this was Oat leaf 7, which showed a gradual linear increase rather than a rapid increase in δD during the stabilisation phase. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber and the control leaves showed an increase in δD over time, reaching between 18.2‰ and 49.1‰, suggesting a change in environmental conditions. However, both the labelled and control leaves were found to be extremely variable due to frequent fluctuations, resulting in an unclear trend overall (Figure 3.10E and Figure 3.12E; Appendix).

The leaf water oxygen isotope composition calculated from the transpired water vapour composition for the leaf portion inside the distal chamber when the basal leaf chamber was exposed to the vapour label showed a gradual increase in $\delta^{18}O$ over time after the label was applied, reaching between 45.6‰ and 56.4‰. The observed increase in leaf water $\delta^{18}O$ again indicates a carry-forward of the labelled water along the leaf (Figure 3.10D; Appendix). The control leaves showed a smaller gradual increase of leaf water $\delta^{18}O$ over time compared to the labelled leaves (Figure 3.12D; Appendix).

The calculated leaf water hydrogen isotope composition for most ^{18}O-labelled leaves showed a gradual increase of δD over time despite fluctuations, reaching between 132.8‰ and 155.8‰ (except for Oat leaf 3) (Figure 3.10F; Appendix). The δD of leaf water for the majority of control leaves also showed an increase over time with minor fluctuations (except for Oat leaf 7) (Figure 3.12F; Appendix). The increase in δD for leaf water across all observations suggested a change in environmental conditions and was generally found to be less significant for the control leaves compared to the labelled leaves (except for Oat leaf 8).

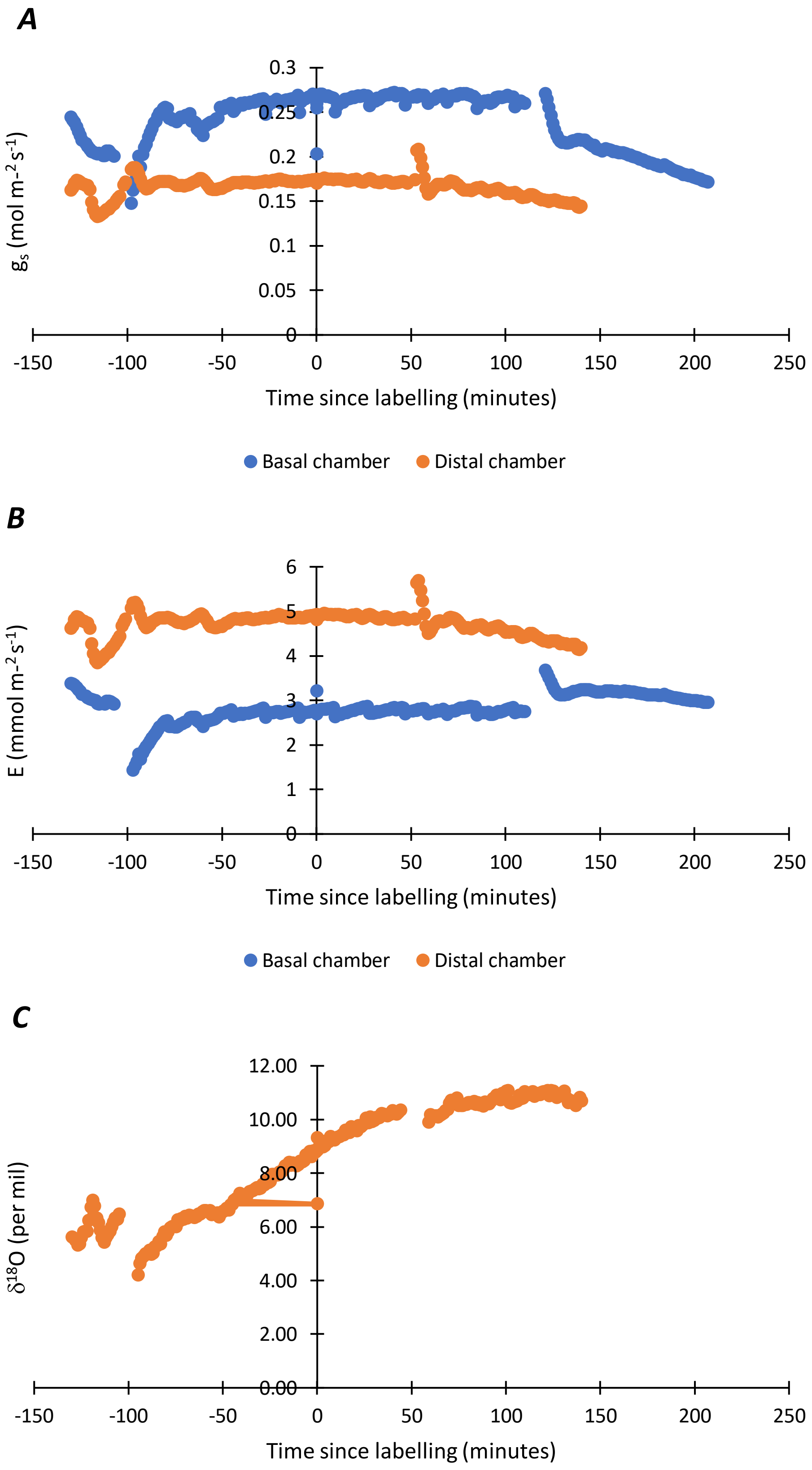

Figure 3.9 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 2 (label).

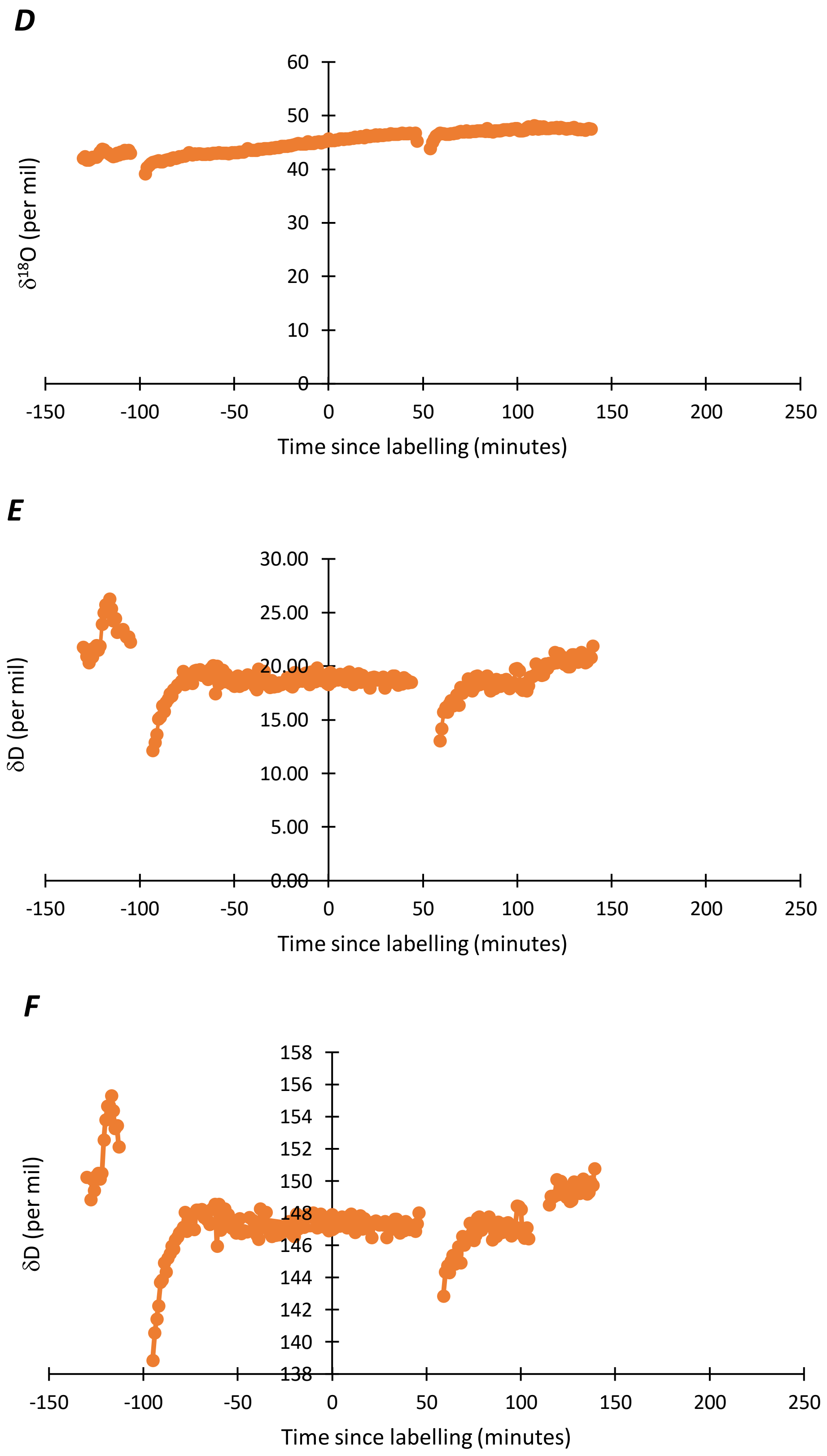

Figure 3.10 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 2 (label).

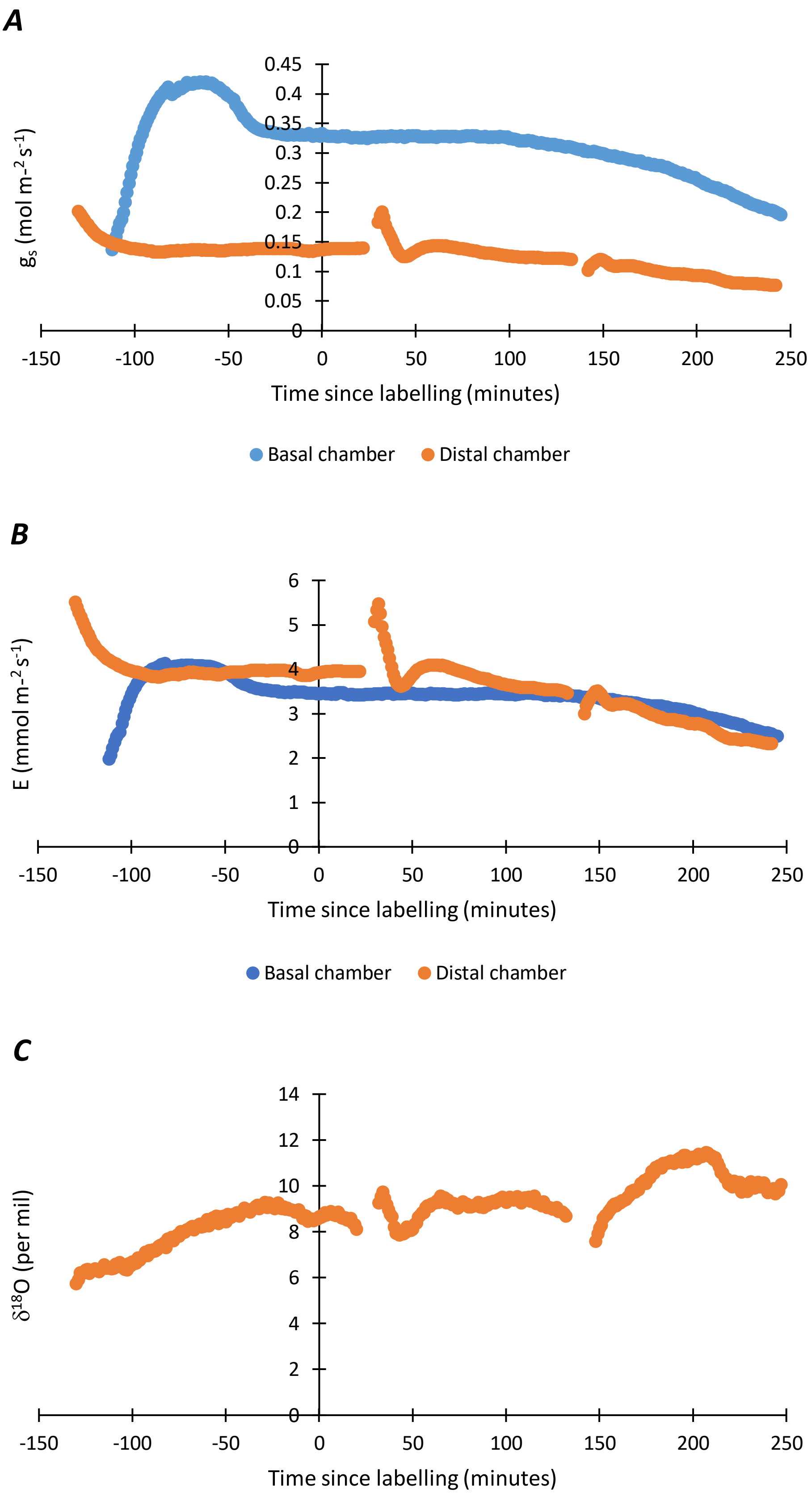

Figure 3.11 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 6 (control).

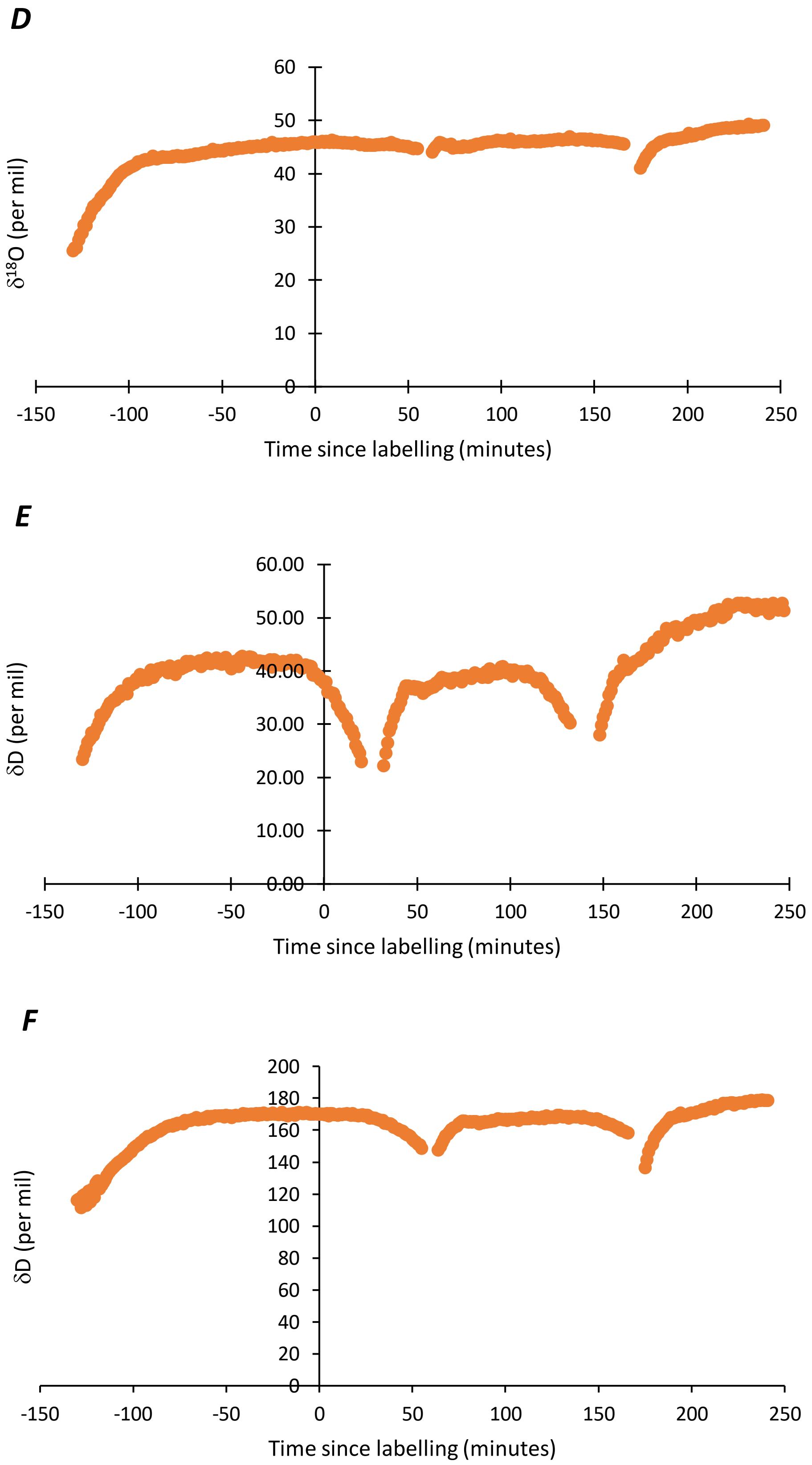

Figure 3.12 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 6 (control).

3.1.3.1 Comparison of changes in isotope composition in labelled and control leaves

The labelled leaves showed a notable change in δ^{18}O for both transpired vapour and calculated leaf water between the start and end values, although none of the changes was found to be statistically significant. The control leaves generally showed a decrease or smaller increase in δ^{18}O for transpired vapour and calculated leaf water and were also not statistically significant. The difference in δ^{18}O was positive for both the labelled and control leaves (except for Oat leaf 6). However, the labelled leaves showed a greater increase compared to the control leaves, indicating the presence of the label in the distal region of the leaf. The change in δD for transpired vapour and leaf water in both the labelled and control leaves was variable, with both small increases or decreases over time, none of which were statistically significant (Table 3.3).

Table 3.3 Changes in transpired vapour and leaf water isotope composition (‰) for Oat

	Plant number	**Label (Oat)**				**Plant number**	**Control (Oat)**			
		$\delta^{18}O_{trans}$	δD_{trans}	$\delta^{18}Oe$	δDe		$\delta^{18}O_{trans}$	δD_{trans}	$\delta^{18}Oe$	δDe
Start	**3**	7.5	38.2	41.6	241.5	**5**	8.7	40.0	43.4	243.8
	4	8.5	19.1	42.8	222.3	**6**	10.8	43.8	45.4	247.5
	5	3.9	-2.0	37.4	201.5	**7**	0.4	-31.4	33.8	169.5
End	**3**	27.2	39.3	218.8	27.2	**5**	10.1	43.4	45.4	247.6
	4	10.7	18.3	45.2	221.9	**6**	9.6	37.3	44.3	241.0
	5	16.1	26.1	51.4	230.5	**7**	5.8	21.7	40.8	226.1
Difference	**3**	17.8	1.1	177.2	-214.3	**5**	1.4	3.4	1.9	3.8
	4	2.2	-0.7	2.4	-0.3	**6**	-1.1	-6.6	-1.0	-6.5
	5	12.2	28.1	14.0	28.9	**7**	5.4	53.1	7.0	56.6
Average of differences		11.6 ± 4.8	8.8 ± 9.7	39.5 ± 30.2	-34.5 ± 53.6	**-**	1.9 ± 1.9	16.6 ± 18.5	1.5 ± 3.2	10.0 ± 26.0
***P*-value**		0.135	0.728	0.280	0.497	**-**	0.135	0.728	0.280	0.497

3.1.4 Sunflower

Among the sunflower leaves, stomatal conductance was found to be lower in the distal chamber in contrast to the basal chamber, for both the labelled and control leaves, with a 19% difference (Figure 3.13A and Figure 3.15A; Appendix). Transpiration rates were seen to be lower in the basal chamber with more minor fluctuations compared to the distal chamber, with a 30% difference for both labelled and control leaves (Figure 3.13B and Figure 3.15B; Appendix). Note that the transpiration rates were lower in the basal chambers compared to the distal chambers for all sunflower leaves due to the higher vapour pressure of the airstreams entering the basal chambers (i.e., water vapour was removed from the inlet air streams for the distal chambers). Across all observations, the measured stomatal conductance and transpiration rates showed a gradual decreasing trend over time for most of the labelled and control leaves. However, transpiration rates showed an increasing trend in the basal chamber for Sunflower leaves 2, 3, and 5 (Appendix).

Both labelled and control leaves showed a rapid increase before approaching a constant δ^{18}O of transpired vapour between -3.0‰ and -1.5‰ during the stabilisation phase with tap water in the bubbler. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber showed a small gradual increase in δ^{18}O over time, reaching between -1.7‰ and 0.0‰ (except for Sunflower leaf 2) (Figure 3.13C; Appendix). As similar increases were observed in the control leaves, sunflower does not show evidence of enrichment (Figure 3.15C; Appendix).

The labelled and control leaves showed a rapid increase before approaching a constant δD of transpired vapour between -22.3‰ and -13.8‰ during the stabilisation phase with tap water in the bubbler. The transpired vapour from the distal chamber of the leaves exposed to the ^{18}O-label in the basal chamber, and the control leaves showed a small gradual increase in δD over time, reaching between -20.3‰ and -11.5‰, suggesting a change in environmental conditions within the distal chamber during the experiment, consistent with changes in stomatal conductance and transpiration rate (Figure 3.14E and Figure 3.16E; Appendix).

The leaf water oxygen isotope composition calculated from the transpired water vapour composition for the leaf portion inside the distal chamber when the basal leaf chamber was exposed to the vapour label showed a small gradual increase in δ^{18}O over time after the label was applied, reaching between 26.5‰ and 31.7‰ (Figure 3.14D; Appendix).

However, the control leaves also showed a small gradual increase in leaf water $\delta^{18}O$, again showing no evidence of enrichment carry-forward of the label for sunflower (Figure 3.16D; Appendix).

The calculated leaf water hydrogen isotope composition of both the ^{18}O-labelled and control leaves showed a small gradual increase of δD over time, reaching between 98.2‰ and 112.5‰, consistent with changes in environmental conditions within the distal chamber (Figure 3.14F and Figure 3.16F; Appendix).

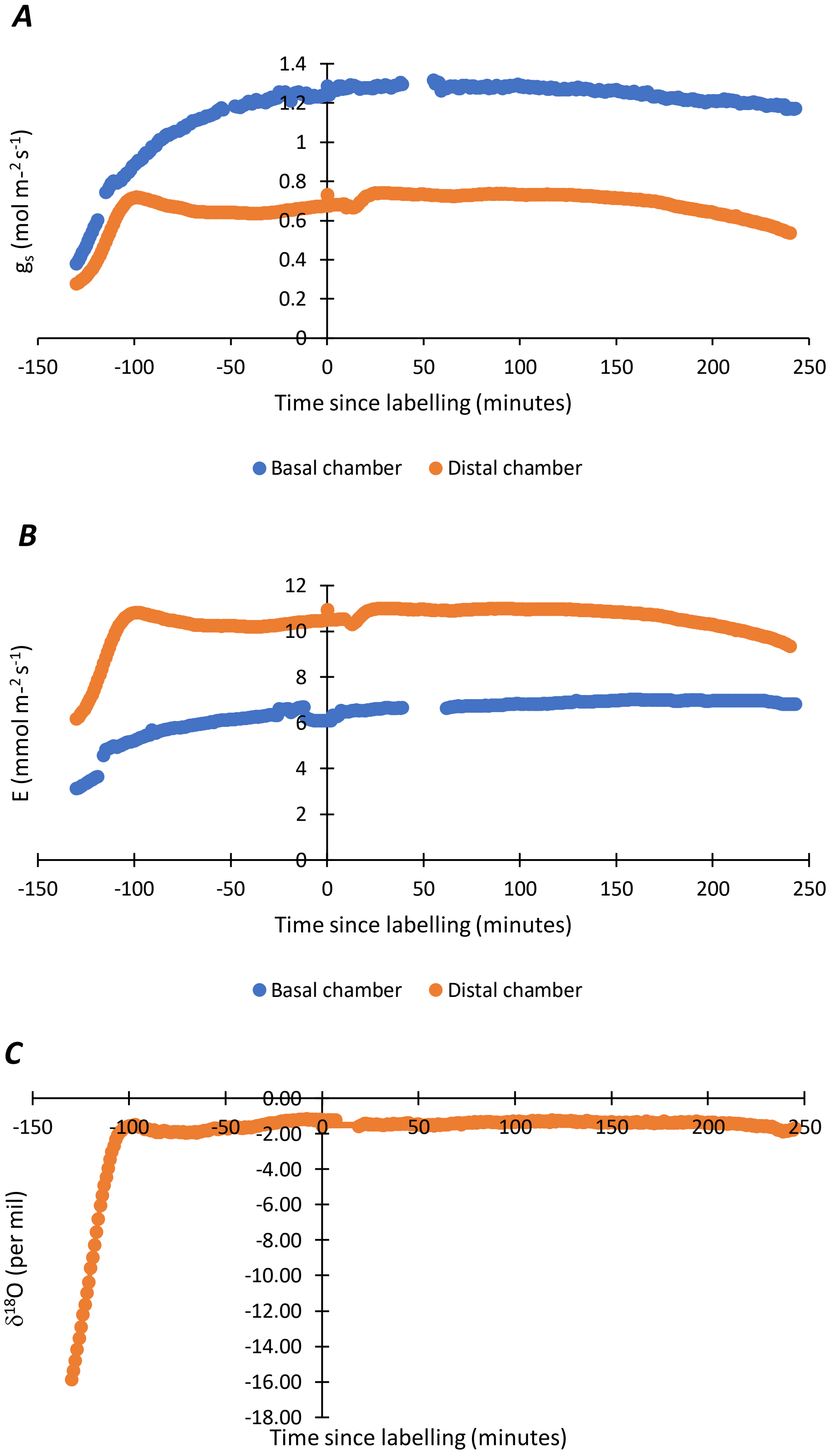

Figure 3.13 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Sunflower plant 2 (label).

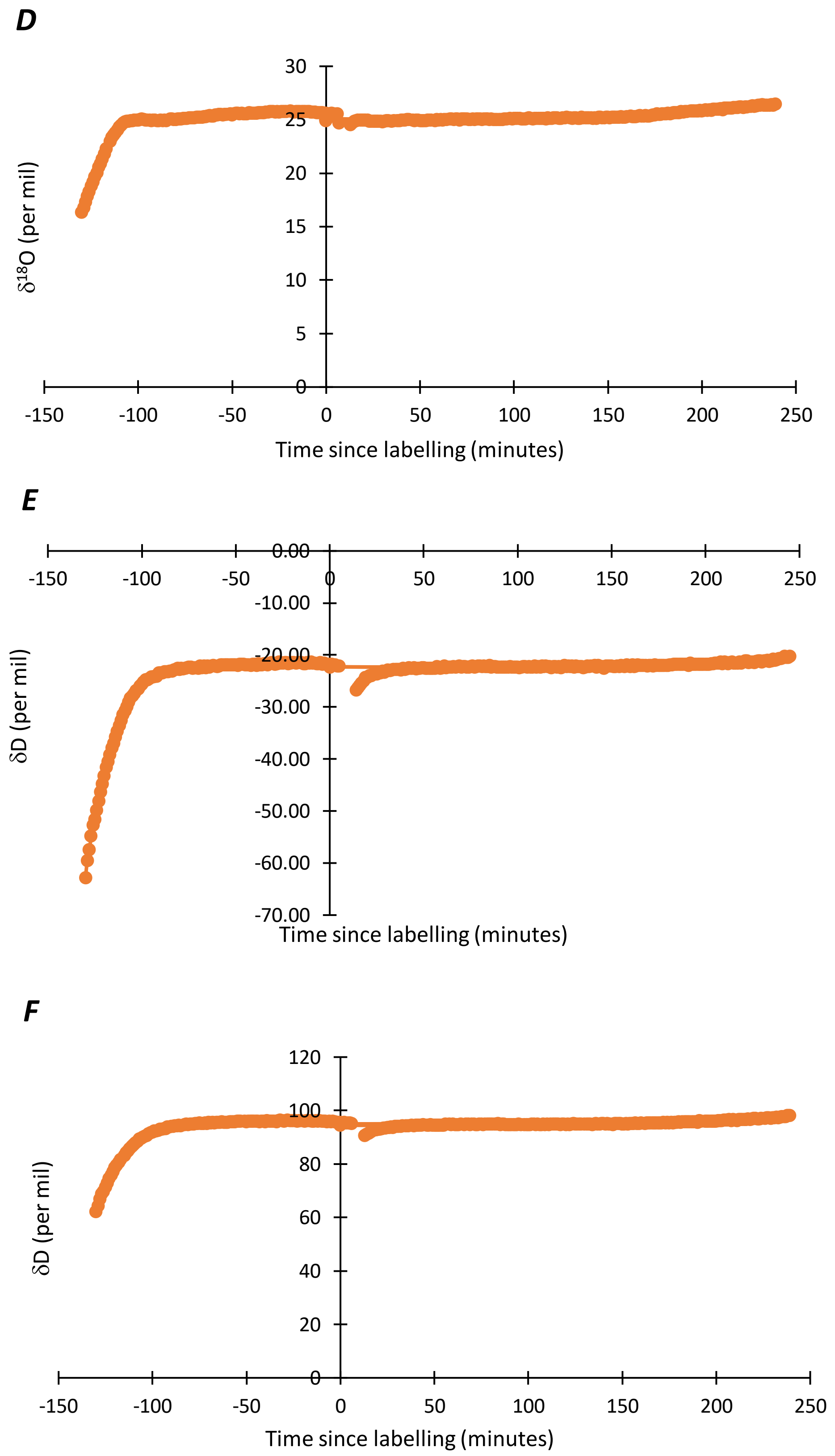

Figure 3.14 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Sunflower plant 2 (label).

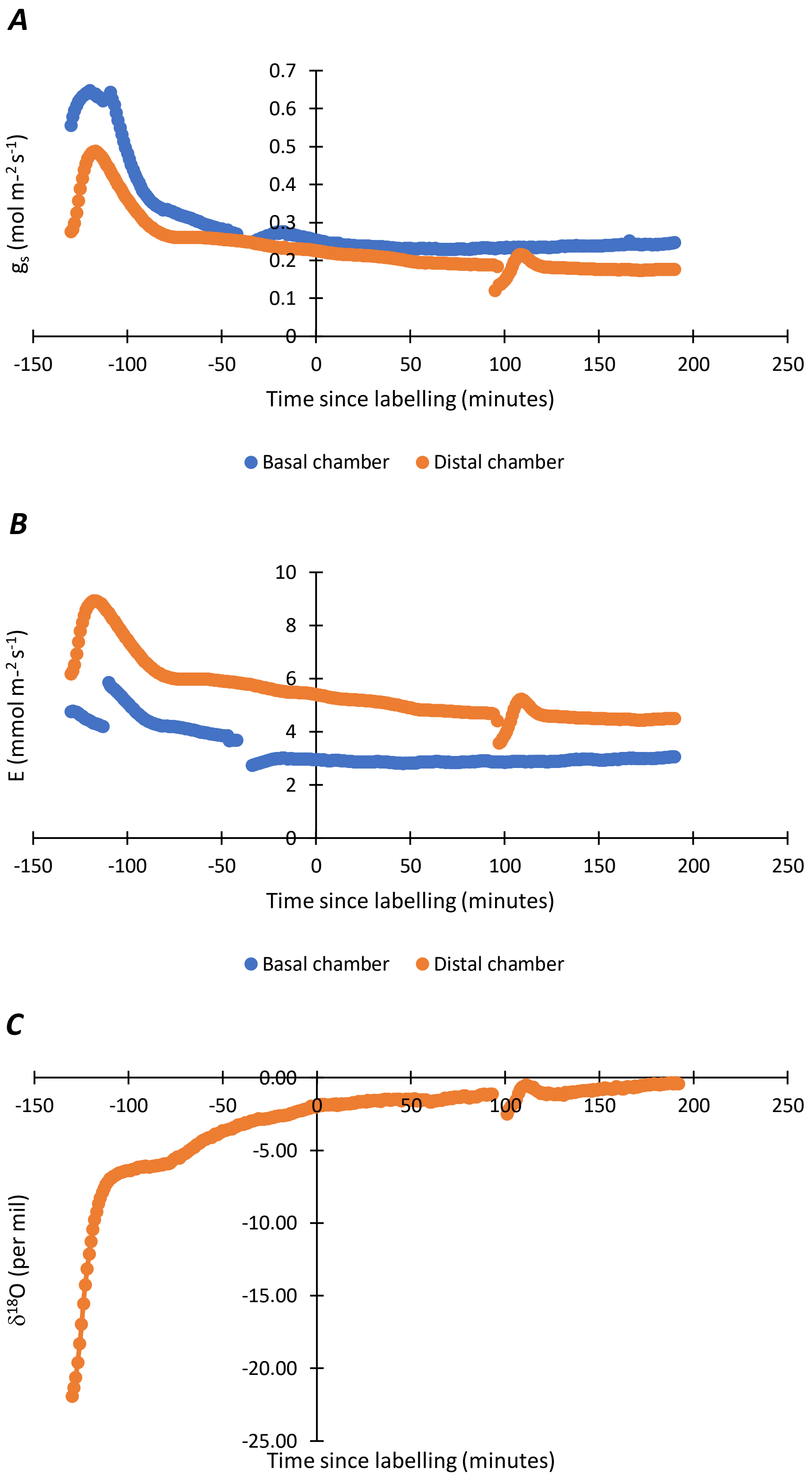

Figure 3.15 Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Sunflower plant 5 (control).

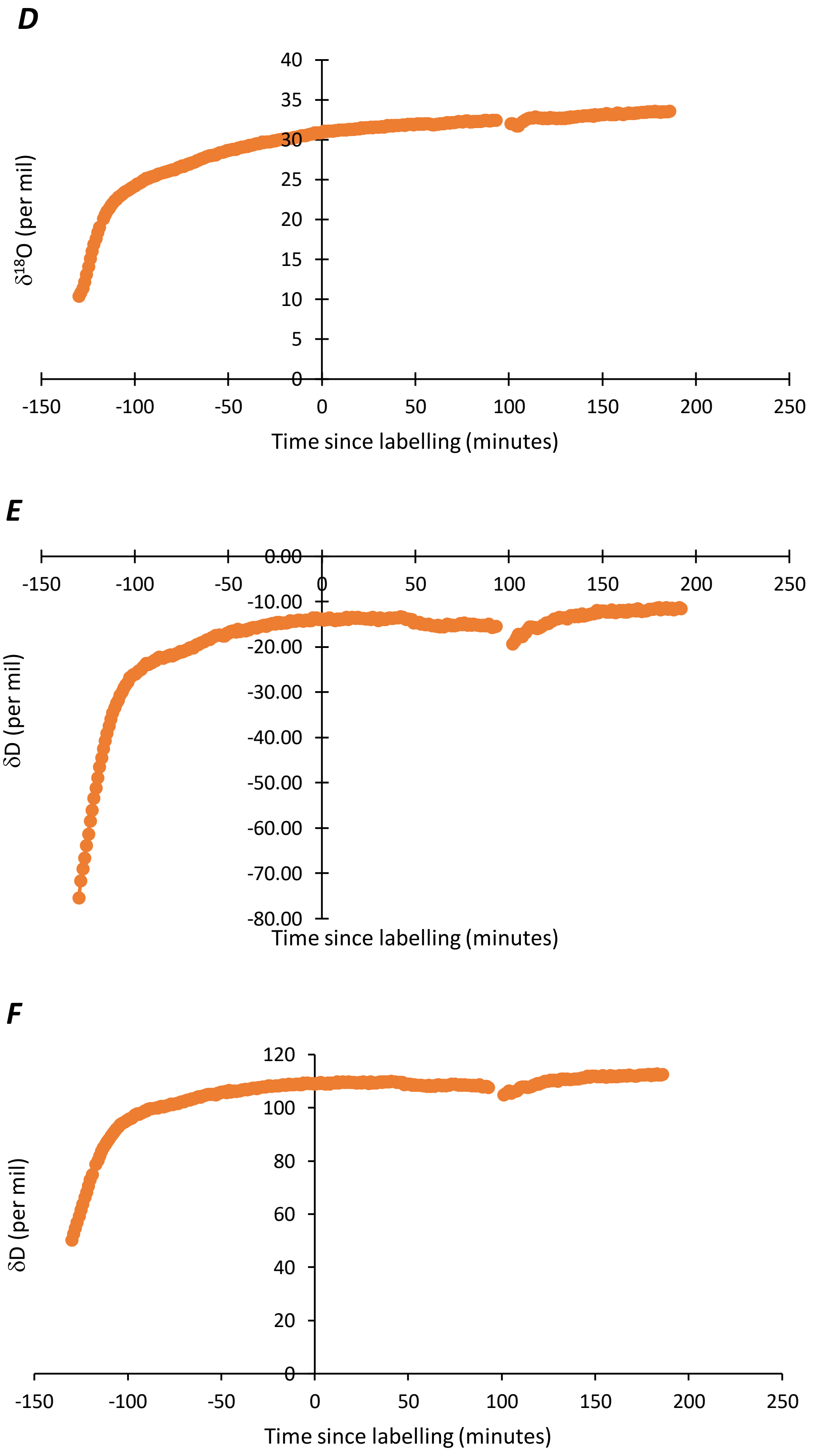

Figure 3.16 Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Sunflower plant 5 (control).

3.1.4.1 Comparison of changes in isotope composition in labelled and control leaves

The labelled and control leaves had a small change in δ^{18}O for both transpired vapour and calculated leaf water between the start and end values, none of which were found to be statistically significant. Most of the difference in δ^{18}O for both the labelled and control leaves was positive (except Sunflower leaf 2). Similarly, δD for transpired vapour and calculated leaf water increased for both the labelled and control leaves, with a greater increase displayed in the control leaves (Table 3.4). Taken together, these results indicate that the $H_2{}^{18}O$ label was not carried from the basal to the distal chamber in sunflower leaves.

Table 3.4 Changes in transpired vapour and leaf water isotopes (‰) for Sunflower.

	Plant number	**Label (Sunflower)**				**Plant number**	**Control (Sunflower)**			
		$\delta^{18}O_{trans}$	**δD_{trans}**	**$\delta^{18}Oe$**	**δDe**		**$\delta^{18}O_{trans}$**	**δD_{trans}**	**$\delta^{18}Oe$**	**$\delta^{18}De$**
Start	**1**	-2.3	-21.0	25.4	176.5	**4**	-2.6	-19.4	25.2	178.2
	2	-1.2	-21.5	23.8	173.4	**5**	-2.2	-14.0	28.7	186.3
	3	-3.0	-20.6	23.2	161.5	**6**	-2.1	-15.4	26.6	182.9
End	**1**	-2.0	-20.0	28.3	179.8	**4**	-0.3	-14.3	27.5	183.3
	2	-1.4	-21.8	23.6	173.2	**5**	-0.4	-11.5	31.6	189.8
	3	-1.8	-18.0	27.3	180.9	**6**	-0.9	-12.0	28.6	187.0
Difference	**1**	0.2	1.0	2.8	3.4	**4**	2.3	5.1	2.3	5.1
	2	-0.2	-0.3	-0.1	-0.2	**5**	1.8	2.5	2.9	3.5
	3	1.2	2.6	4.1	19.4	**6**	1.2	3.4	2.0	4.1
Average of differences		0.4 ± 0.4	1.1 ± 0.9	1.5 ± 0.9	2.7 ± 1.5	**-**	1.8 ± 0.3	3.6 ± 0.8	2.4 ± 0.2	4.2 ± 0.5
Standard error of differences		0.060	0.092	0.405	0.390	**-**	0.060	0.092	0.405	0.390

3.1.5 Comparison between species

The change in $\delta^{18}O$ followed the same trends for both transpired vapour and calculated leaf water isotope composition. Oat had the largest increase in $\delta^{18}O$ for both the transpired vapour of 11.6 ± 4.8‰ and calculated leaf water of 39.5 ± 30.2‰ (Table 3.5). Corn showed the second highest increase in $\delta^{18}O$ with an average difference of 8.0 ± 0.6‰ for transpired vapour and 8.6 ± 0.6‰ for calculated leaf water. Foxglove showed the next highest increase for $\delta^{18}O$ with an average difference of 5.6 ± 0.8‰ for transpired vapour and 5.8 ± 0.8‰ for calculated leaf water. Sunflower showed the least amount of change in $\delta^{18}O$ as increases in the label were very small or similar to the control group, with an average difference of 0.4 ± 0.4‰ for transpired vapour and 1.5 ± 0.9‰ for calculated leaf water. The increases in $\delta^{18}O$ for corn and foxglove were found to be statistically significant ($\delta^{18}O_{trans}$ P=0.003*** and P=0.01**; $\delta^{18}O_e$ P=0.01** and P=0.02**), while oat and sunflower were not found to be statistically significant (Table 3.1; Table 3.2; Table 3.3; Table 3.4). Based on the average differences for $\delta^{18}O$ in transpired vapour and calculated leaf water, corn presented the most significant evidence for carry-forward of the $H_2{}^{18}O$ label, followed by foxglove. Oat presented evidence for carry-forward of the $H_2{}^{18}O$ label for two out of three labelled leaves, although it was not statistically significant and was unreliable due to a large standard error. Meanwhile, sunflower did not show any significant evidence for carry-forward of the $H_2{}^{18}O$ label.

The transpired vapour hydrogen isotope composition showed foxglove as having the largest increase in δD with an average difference of 16.7 ± 4.6‰ for transpired vapour. Oat showed the second largest increase in δD for transpired vapour with an average difference of 8.8 ± 9.7‰. Sunflower showed the next largest increase in δD for transpired vapour with an average difference of 1.1 ± 0.9‰. Finally, corn showed the smallest increase in δD for transpired vapour with an average difference of 0.4 ± 1.4‰ (Table 3.5). Similarly, foxglove also showed the largest increase in δD for calculated leaf water with an average difference of 17.0 ± 4.8‰.

Meanwhile, sunflower was seen to have the second largest increase for calculated leaf water with an average difference of 2.7 ± 1.5‰. Corn had the next largest increase for calculated leaf water with an average difference of -0.4 ± 0.8‰. However, oat deviated from the trend for calculated leaf water as it showed a large decrease in δD with an average difference of -34.5 ± 53.6‰. Overall, none of the changes in δD for transpired vapour or leaf water isotope composition was found to be statistically significant (Table 3.1; Table 3.2; Table 3.3; Table 3.4).

Table 3.5 Change in transpired vapour and leaf water isotopes (‰) for all species.

Species	**Group**	**Average of differences**			
		$\delta^{18}O_{trans}$	δD_{trans}	$\delta^{18}Oe$	δDe
Corn	**Label**	8.0 ± 0.6	0.4 ± 1.4	8.6 ± 0.6	-0.4 ± 0.8
	Control	0.1 ± 1.1	1.3 ± 1.9	0.8 ± 1.2	2.5 ± 2.1
Foxglove	**Label**	5.6 ± 0.8	16.7 ± 4.6	5.8 ± 0.8	17.0 ± 4.8
	Control	1.7 ± 0.4	6.9 ± 0.8	1.8 ± 0.7	7.0 ± 0.6
Oat	**Label**	11.6 ± 4.8	8.8 ± 9.7	39.5 ± 30.2	-34.5 ± 53.6
	Control	1.9 ± 1.9	16.6 ± 18.5	1.5 ± 3.2	10.0 ± 26.0
Sunflower	**Label**	0.4 ± 0.4	1.1 ± 0.9	1.5 ± 0.9	2.7 ± 1.5
	Control	1.8 ± 0.3	3.6 ± 0.8	2.4 ± 0.2	4.2 ± 0.5

3.1.6 Net versus one-way fluxes of water into leaves

The calculated w_a/w_i values for the basal portion of the labelled leaves showed large one-way fluxes of vapour into the leaf for each species and were significantly different between species. Oat and sunflower had the highest ratio of w_a/w_i with 0.74 and 0.73 entering through the vapour phase, respectively, followed by corn with a w_a/w_i of 0.67, while foxglove had the lowest w_a/w_i with only 0.57 entering through the vapour phase. The one-way flux of vapour into the leaf was significantly different between species, as foxglove was significantly lower than corn, and corn was significantly lower than sunflower. No significant difference was seen between the one-way fluxes for sunflower and oat (see Table 3.6).

Table 3.6 The ratio of atmospheric to intercellular water vapour mole fraction (w_a/w_i) for all labelled leaves for the four species. Note that w_a/w_i is the proportion of water entering the leaf in the vapour phase.

Species	**Leaf**	**w_a/w_i**
Corn	1	0.67
	2	0.63
	8	0.60
	Average	0.63 ± 0.02
Oat	3	0.69
	4	0.74
	5	0.79
	Average	0.74 ± 0.03
Sunflower	1	0.72
	2	0.76
	3	0.70
	Average	0.73 ± 0.02
Foxglove	1	0.57
	3	0.58
	5	0.51
	Average	0.55 ± 0.02

3.2 Experiment Two

3.2.1 Spatial variation in leaf water isotopes

δ^{18}O leaf water for Foxglove leaf 1 was seen to be highest at the second position up from the base at 20.6‰ and decreased along the leaf towards the tip. The spatial pattern for δ^{18}O leaf water observed in Foxglove plant 1 does not suggest progressive enrichment as it was seen to be lowest at the fourth position up from the base at 17.7‰ rather than at the base of the leaf as expected (Figure 3.17).

δ^{18}O leaf water for Foxglove leaf 2 was seen to be highest at the base on the right side of the leaf at 32.1‰ and lowest at the tip of the leaf at 23.2‰. The left side of the leaf showed an increase in ^{18}O from the base at 23.3‰ to the third position at 26.1‰ before decreasing upon reaching the tip. The spatial patterns displayed for Foxglove plant 2 do not suggest progressive enrichment, as δ^{18}O was seen to be lowest at the tip of the leaf (Figure 3.18).

δ^{18}O leaf water for Foxglove leaf 3 was seen to be highest at the third position on the left side of the leaf at 33.6‰. An increase in δ^{18}O was seen between the second and third position on the left side of the leaf but decreased to 30.1‰ at the tip. The spatial pattern seen in Foxglove plant 3 does not suggest enrichment, as δ^{18}O decreased towards the tip on the left side of the leaf (Figure 3.19).

δ^{18}O leaf water for Foxglove leaf 4 was seen to be highest at the tip at 32.3‰ and lowest at the second position on the right side of the leaf at 23.5‰. Both sides of the leaf showed a decrease between the base and second position, suggesting no evidence of progressive enrichment despite δ^{18}O being the highest at the tip (Figure 3.20).

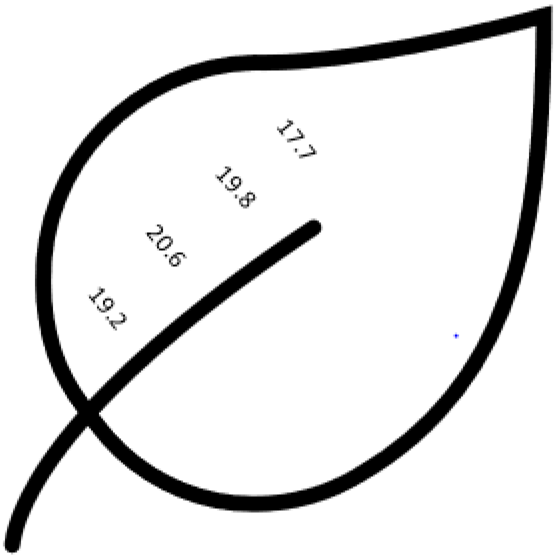

Figure 3.17 Spatial variation of leaf water $\delta^{18}O$‰ for Foxglove leaf 1.

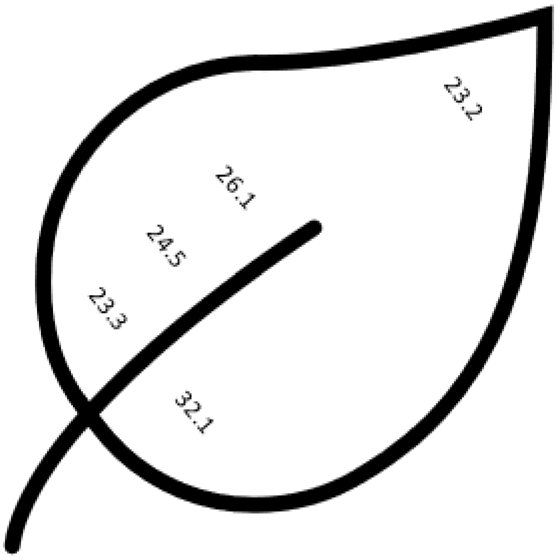

Figure 3.18 Spatial variation of leaf water $\delta^{18}O$‰ for Foxglove leaf 2.

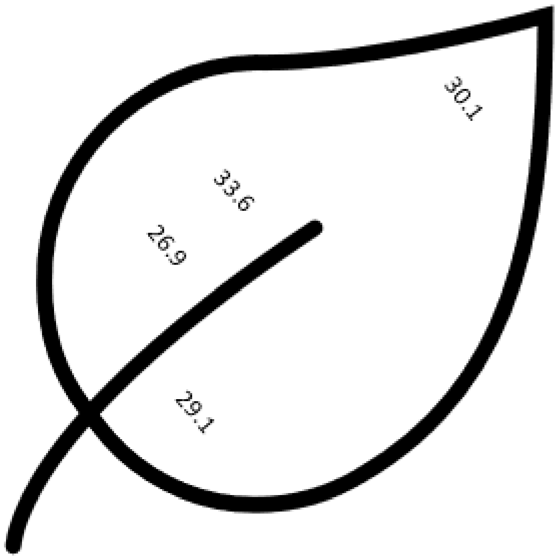

Figure 3.19 Spatial variation of leaf water δ^{18}O‰ for Foxglove leaf 3.

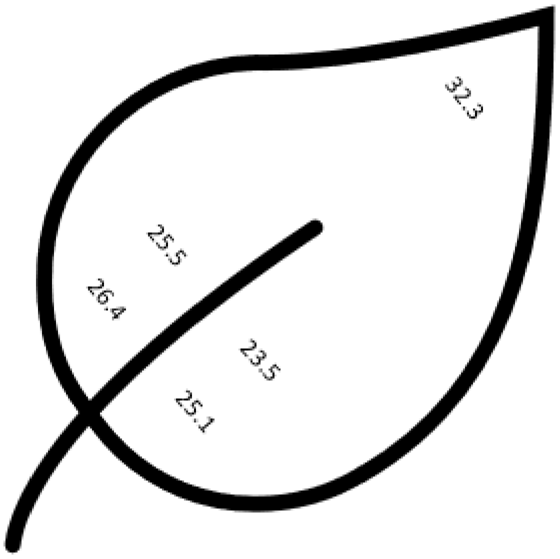

Figure 3.20 Spatial variation of leaf water δ^{18}O‰ for Foxglove leaf 4.

3.3 Experiment Three

3.3.1 Transpiration rate vs. fractional difference

In foxglove leaves, the significant positive relationship seen between transpiration rate and fractional difference (*P*=0.018) presents evidence for enrichment via a Péclet effect in foxglove. The transpiration rate showed a statistically significant positive correlation with the fractional difference ($1-\Delta_L/\Delta_e$). Transpiration rate was seen to explain 41% of the variation in the fractional difference (R^2=0.41) (Figure 3.21).

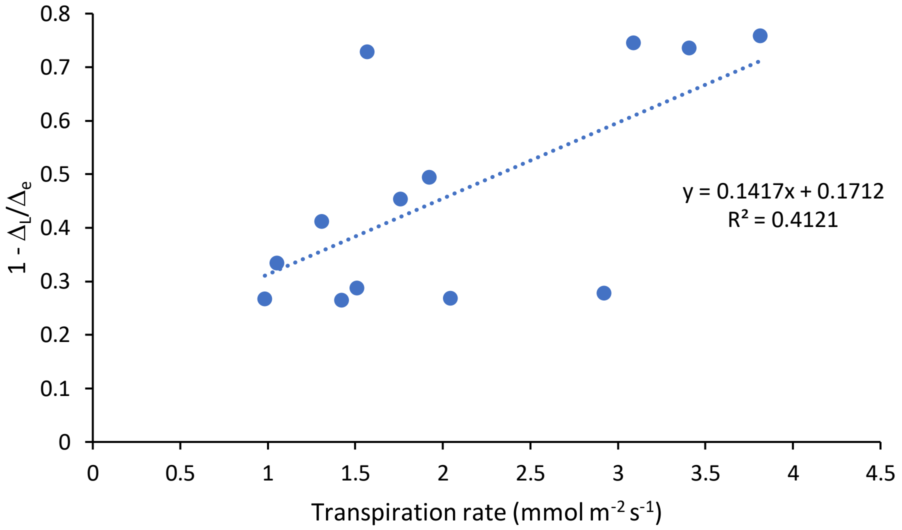

Figure 3.21 Linear regression of transpiration rate vs. fractional difference (*P*=0.018).

Chapter 4

Discussion

4.1 Gas exchange

The measured parameters for gas exchange used in the current study were stomatal conductance and transpiration rates, which, when compared with plants of the same or similar species in previous studies, were shown to be within the normal range for leaves. In the current study, stomatal conductance for corn varied between 0.20 and 0.10 mol m^{-2} s^{-1} and transpiration rates varied between 1.52 and 4.95 mmol m^{-2} s^{-1} compared to 0.14 mol m^{-2} s^{-1} and 3.46 mmol m^{-2} s^{-1}, respectively in studies by Khan et al. (2003) and Anjum et al. (2011). Oat was found to have stomatal conductance of between 0.07 and 0.34 mol m^{-2} s^{-1} and a transpiration rate between 2.32 and 6.20 mmol m^{-2} s^{-1} which appears to fit within the normal range when compared to values of 0.15 mol m^{-2} s^{-1} and 2.93 mmol m^{-2} s^{-1}, respectively from a study by Shangguan et al. (2000) on gas exchange in wheat. Foxglove leaves measured here showed stomatal conductance between 0.04 and 0.12 mol m^{-2} s^{-1} and transpiration rates between 0.26 and 2.12 mmol m^{-2} s^{-1}, which, when compared to that of bean at 0.20 mol m^{-2} s^{-1} and 1.91 mmol m^{-2} s^{-1}, respectively in a study by Rodrigues et al. (2015) appears normal for transpiration rates. Sunflower leaves had a stomatal conductance of between 0.17 and 1.25 mol m^{-2} s^{-1} and a transpiration rate of between 3.07 and 10.48 mmol m^{-2} s^{-1} in the current study, which fits within the normal range when compared to values of 0.50 mol m^{-2} s^{-1} and 5.57 mmol m^{-2} s^{-1}, respectively from a study by Simonin et al. (2015). These comparisons show that measurements of stomatal conductance and transpiration rate for the species tested in the current study were largely within the normal range and showed appropriate responses for healthy leaves.

4.2 Isotopically labelled water vapour allows for exploration of hydraulic pathways in leaves

Exploring changes in leaf water isotopic composition through vapour isotope labelling has been suggested in previous studies by Barbour et al. (2021) but has never been tested experimentally until now. The current study investigates leaf hydraulic pathways by introducing an ^{18}O-label through water vapour. The experiments shown here were designed to test the relevance of Péclet-type patterns of leaf water isotopic enrichment in both monocot and dicot species and to help answer the question of whether Péclet effect enrichment gradients extend back into the veins to be carried forward to distal portions of leaves (Helliker & Ehleringer, 2000). It was hypothesised that progressive enrichment from basal to distal portions of leaves is due to diffusion of enriched mesophyll water into the xylem, associated with a localised Péclet effect within the vascular tissue (Song et al., 2015a). Following Gan et al. (2003), Péclet effects and associated isotope gradients within mesophyll cells are distinguished from Péclet effects and associated isotope gradients within cells in the vascular tissue. The former I call mesophyll Péclet effects, and these may result in observed positive relationships between transpiration rate and the fractional difference between bulk leaf water, and Craig-Gordon predicted isotope compositions (as described by (Barbour et al., 2000; Flanagan et al., 1991). The latter I call vascular Péclet effects (although not longitudinal, as Gan does; both mesophyll and vascular Péclet effects are radial in orientation) and may result in spatial patterns of increasing enrichment from the base to the tip and outside of leaves (Song et al., 2015a; Wang & Yakir, 1995).

The results of this study present direct experimental evidence, showing that the isotopic composition of water within mesophyll cells influences the transpired vapour isotope composition in distal portions of leaves in three out of four species. This finding is important as it highlights differences in hydraulic pathways and design between species. Further, these findings may help us understand the generation of gradients for isotope enrichment via the Péclet effect, particularly across different tissues, such as the vascular and mesophyll tissues.

The use of vapour isotope labelling in the current experiments also presented an opportunity to investigate the contributions of different phases, such as gas and liquid, to water transport within leaves (Farquhar & Cernusak, 2005). The contribution of each phase was evaluated by calculating the ratio of atmospheric to intercellular water vapour mole fraction (w_a/w_i) for the basal portion of leaves exposed to the ^{18}O label. The w_a/w_i was calculated for leaves of each species and allowed the amount of water entering the leaf through the vapour or liquid phase to be gauged and net versus one-way fluxes compared. The resulting w_a/w_i ratios varied between species, but all showed that a significant (>50%) proportion of water entered the leaf in the vapour phase. Notably, sunflower had the second highest proportion of water entering the leaf in the vapour phase in the basal leaf portion, despite showing no evidence of the label in the transpired vapour from the distal leaf portion. This finding is significant as the lack of change in isotopic composition even with a large one-way vapour flux in sunflower highlights a difference in hydraulic design between the species measured. The lack of change seen in δD composition amongst all species tested also supports the changes seen in $\delta^{18}O$ as this provides evidence that changes within the species tested were not due to environmental conditions, thus acting as a secondary control for comparison.

4.3 Hydraulic pathways and the Péclet effect in monocot leaves

The current study found a change in $\delta^{18}O$ of transpired vapour and calculated leaf water seen in Experiment One for the labelled leaves of both monocot species: corn and oat. The change in $\delta^{18}O$ was found to be statistically significant for corn but was statistically non-significant for oat. The non-significant change in $\delta^{18}O$ for oat can be explained by the fact that only two out of three replicates for the labelled leaves produced reliable data in Experiment One. The unreliable replicate for oat was due to the bubbler containing the ^{18}O-label becoming disconnected during the measurement period and exposing the leaf to the uncontrolled environment within the room. These results show that the isotopic composition of the water within the mesophyll influences the transpired vapour isotopic composition in the distal portion of both corn and oat leaves, thus supporting the hypothesis and demonstrating the relevance of at least a vascular Péclet effect to isotopic enrichment within leaves. The progressive enrichment of mesophyll and xylem water along leaves has been thought to be possible due to backwards diffusion into the xylem, as it would allow enriched water such as the ^{18}O-label from the base of the leaf to enter the xylem and be carried forward to the distal part of the leaf where it can be seen in measurements of transpired vapour such as shown in this study (Gan et al., 2003; Helliker & Ehleringer, 2000). To reach the xylem from the leaf interior in the vapour phase, the $H_2{}^{18}O$ label must diffuse through the mesophyll. This is similar to a naturally transpiring, unlabelled leaf in which evaporative enrichment occurs at the evaporating surfaces within the leaf interior. The vapour labelling experimental protocol employed here does not provide evidence for or against a mesophyll Péclet effect but does demonstrate that water isotopologues can diffuse backwards into the xylem.

However, the occurrence of isotopic enrichment through the backwards diffusion within either the mesophyll cells or the vascular cells depends on the velocity of convection relative to backwards diffusion and the effective path length over the transport pathway in question. The effective path length can be influenced by the presence of resistances within the leaf, such as whether the leaf contains bundle sheath cells or not and the possible levels of tortuosity, i.e., the number of curves and/or turns, and connectivity found within and between mesophyll cells. The velocity of water movement or transport is influenced by the concentration of water and the rate of transpiration, which can vary with environmental conditions such as temperature, relative humidity, and water availability (Gan et al., 2003; Helliker & Ehleringer, 2000). Additionally, the amount of water within the mesophyll may be influenced by the contribution of different phases of water, such as vapour and liquid water entering the leaf, also referred to as the number of one-way fluxes of water vapour into the leaf, as shown by w_a/w_i (Farquhar & Cernusak, 2005). Corn and oat showed large one-way vapour fluxes compared to the net flux of water into the leaf. The high one-way vapour fluxes offer more support to the influence of mesophyll water on the isotopic composition of the distal portion of the leaf as it shows more water entering through the mesophyll in the vapour phase rather than through the veins in the liquid phase (Farquhar & Cernusak, 2005).

The enrichment of leaf water via the Péclet effect, as shown here through calculated leaf water values for both corn and oat, has been supported by findings of spatial variation in δ^{18}O of leaf water. A previous study by Song and Barbour (2016) found an increase in δ^{18}O leaf water along the blade of a corn leaf, with a strong pattern of progressive enrichment observed. The results showed a statistically significant increase in δ^{18}O leaf water from the base to the tip of the corn leaf, supporting the hypothesis that leaf water enrichment may be carried-forward from basal to distal regions of corn leaves seen in our current study. While very little could be found on leaf water enrichment in oat, evidence of longitudinal enrichment has been seen in similar species such as barley which showed an increase in δ^{18}O leaf water from the base to tip, supporting the enrichment observed here for oat (MM Barbour pers. comm.).

However, neither corn nor species similar to oat, such as wheat, have been found to have a significant relationship between transpiration rate and the fractional difference between bulk leaf water and modelled Craig-Gordon water (Barbour et al., 2021). Barbour et al. (2021) found that wheat showed no significant correlation between transpiration rate and fractional difference, suggesting leaf water enrichment could not occur via the Péclet effect. Corn was also seen to have a non-significant relationship between transpiration rate and fractional difference in previous work (MM Barbour pers. comm.). Although in both cases, the oxygen isotope composition of leaf water was found to be less than the

Craig-Gordon estimate indicating that the Craig-Gordon model alone is insufficient for accurate predictions of isotope composition (Barbour et al., 2021 and MM Barbour pers. Comm). As neither the Péclet effect nor the Craig-Gordon model was found to predict changes in leaf water isotope composition, this indicates the need for a different model, such as the two-pool model suggested by Barbour et al. (2021) based on their work investigating hydraulic design. Despite uncertainties around the correct model for predicting leaf water oxygen isotope enrichment, further support for longitudinal enrichment is offered by previous work on leaf water enrichment in monocot leaves by Gan et al. (2003) and Helliker and Ehleringer (2000), who also found a significant pattern of increasing enrichment from the base to the tip of leaves in bulk leaf water.

To reconcile these seemingly contradictory results, both monocot species appear to have a significant vascular Péclet effect but a non-significant mesophyll Péclet effect (see Table 7).

4.4 The Péclet effect is relevant for only one of two dicot species

An interesting finding from this study was seen in the different appearances of the ^{18}O-label among the dicot species. The results of foxglove are important as they indicate a carry-forward of the ^{18}O vapour label from the basal region in dicot species. The ^{18}O vapour label only appeared in the distal regions of labelled leaves in measurements of transpired vapour and calculated leaf water for foxglove and was not seen in sunflower, suggesting a difference in hydraulic design. However, the presence of a significant vascular Péclet effect in foxglove, as evidenced by the carry-forward of enrichment, was not supported by measurements of spatial variation in δ^{18}O as no clear patterns were observed. The lack of patterns in spatial variation for foxglove leaves could indicate a potential difference in hydraulic conductance and pathways used for water transport between monocot and dicot leaves. As dicot leaves have more complicated patterns of venation compared to the simple parallel venation of monocot leaves such as corn and oat, the higher levels of tortuosity may create a greater resistance in pathways for bulk water transport as it is not straightforward (Barbour et al., 2021; Helliker & Ehleringer, 2000). This idea was supported not only by the lack of spatial variation seen in foxglove leaves but also by sunflower leaves which showed a very small increase in δ^{18}O of leaf water from the centre to the edges of leaves in a previous study by Wang and Yakir (1995). The increase in δ^{18}O in sunflower leaf water was only up to 3.1‰, compared to increases seen in monocot leaves which ranged from 15.0‰ in corn to 25.0‰ in barley (Song & Barbour, 2016; Wang & Yakir, 1995 and MM Barbour pers. comm).

So, although foxglove leaves showed no evidence of spatial variation in enrichment (i.e., limited evidence of a vascular Péclet effect), the presence of a significant positive relationship between transpiration rate and fractional difference demonstrates that a mesophyll Péclet effect model is relevant to foxglove (see Table 4.1). In contrast, sunflower leaves exposed to the ^{18}O vapour label displayed very little change in transpired vapour compared to the control leaves and the observed changes in foxglove. The results of sunflower seen here present no evidence of either a vascular or a mesophyll Péclet effect and agree with the conclusions by Wang and Yakir (1995) in their work on spatial variation, which showed that the Péclet effect model was not relevant to the enrichment of leaf water. Another recent study by Barbour et al. (2021) showed a significant positive relationship between transpiration rate and fractional difference for sunflower, although this result was driven by a single measurement at a low transpiration rate. Meanwhile, a separate experiment displayed a non-significant relationship for transpiration rate versus the fractional difference in the same study, indicating the Péclet effect model is not appropriate for predicting leaf water isotopic enrichment in sunflower, instead suggesting the use of the two-pool model based on their investigation into leaf hydraulic design (Barbour et al., 2021). As the evidence presented in other studies on sunflower points out, there is limited relevance of the Péclet effect for sunflower leaf water enrichment, and no significant changes were seen in results from the current experiments. This, again, suggests a difference in leaf hydraulic design between sunflower and foxglove.

4.5 Why do Péclet-like gradients in $H_2{}^{18}O$ within leaves appear to be relevant in only some species?

A vascular Péclet effect was found to be relevant for the isotopic enrichment of transpired vapour and calculated leaf water in three out of four species tested in the current study: corn, oat and foxglove. Oat showed one of the largest changes in $\delta^{18}O$ for both transpired vapour and calculated leaf water but was found to be non-significant due to an unreliable replicate in the labelled group. Corn showed the next largest increase in $\delta^{18}O$, and the changes were found to be statistically significant for both transpired vapour and calculated leaf water. Foxglove also showed a statistically significant change in $\delta^{18}O$ for transpired vapour and calculated leaf water, although smaller than both monocots. In contrast, sunflower showed no change in $\delta^{18}O$ for either transpired vapour or calculated leaf water, with very little difference seen between the labelled and control leaves, suggesting no evidence of a vascular Péclet effect.

The results of the transpired vapour measurements for both monocot species showed a carry-forward of the ^{18}O vapour label from basal to distal regions of the leaf, with the change in $\delta^{18}O$ demonstrating the relevance of a vascular Péclet effect to isotopic enrichment. A vascular Péclet effect in monocot leaves was supported further by evidence of longitudinal enrichment in similar species shown in previous studies (Song & Barbour, 2016). There was no support for a mesophyll Péclet effect for either monocot species studied here (Barbour et al., 2021).

Foxglove also showed the carry-forward of the ^{18}O vapour label from basal to distal regions of the leaf resulting in enrichment via the vascular Péclet effect. Even though the enrichment seen in foxglove was lower than that of corn, the presence of a mesophyll Péclet effect was supported by a significant positive relationship between transpiration rate and fractional difference, which shows that the Péclet effect model is best for predicting oxygen isotope enrichment in foxglove leaves. However, foxglove showed no evidence of longitudinal enrichment in measurements of spatial variation from the current study suggesting a difference in hydraulic design compared to the monocot leaves.

On the other hand, sunflower showed small increases in $\delta^{18}O$ of leaf water in a study by Wang and Yakir (1995) on spatial variation. Another study on sunflower by Barbour et al. (2021) proposed using the two pool model as it was found to predict the oxygen isotope composition based on the absence of a strong relationship between transpiration rate and fractional difference. The differences between studies for sunflower supports the results of our study, which indicates that a different model is needed for the isotopic enrichment of sunflower leaves, suggesting a difference in leaf hydraulic design. Sunflower appears to have neither a vascular nor a mesophyll Péclet effect.

Current evidence indicates that leaf hydraulic design and its influence on water transport pathways is a key factor in determining the isotope composition of leaf water and should be used to inform the decision of which model most accurately predicts the oxygen isotope composition. Leaf hydraulic design encompasses the variation of anatomy between species and leaves, accounting for venation, tissue connectivity and structure, and the presence of cells such as bundle sheath extensions (Holloway-Phillips et al., 2016). An example of this is shown by the difference in enrichment seen between the monocot and dicot leaves such as corn and foxglove, as foxglove is seen to have lower levels of enrichment and does not display longitudinal enrichment as seen in studies of spatial variation for monocot leaves (Gan et al., 2003; Helliker & Ehleringer, 2000). Monocot leaves have parallel veins that are straightforward, likely allowing for a simpler pathway and thus a higher velocity of water movement (Helliker & Ehleringer, 2000). In contrast, dicot leaves have branching veins in which the amount of turns or tortuosity complicates the pathway, slowing the velocity of water transport (Barbour & Farquhar, 2004; Barbour, 2007; Loucos et al., 2015). This may offer a possible explanation for the lower levels of carry-forward of the $H_2{}^{18}O$ label, and the lack of spatial variation seen in foxglove, as a more complex pathway may cause greater resistance and lead to a slower velocity for water transport thus limiting enrichment.

In monocot leaves such as corn, a simple pathway with a higher density of vascular bundles likely means lower tortuosity over shorter distances, allowing for relatively more backwards diffusion of enrichment within the mesophyll (Loucos et al., 2015). To reconcile the lack of a relationship between transpiration rate and the fractional difference in leaf water enrichment, monocot leaves would appear to have a limited mesophyll Péclet effect (i.e., the mesophyll is at Craig-Gordon enrichment) but a strong vascular Péclet effect.

Additionally, the contribution of different pathways such as apoplastic, symplastic and transcellular to the transpiration flux and their interactions are thought to influence the development of gradients for enrichment within leaves and may also affect the resulting isotope composition. An example of this can be seen in leaf hydraulic design as apoplastic pathways allow the development of isotopic or Péclet type gradients; this is because apoplastic water movement is dominated by bulk flow, and the Péclet effect describes the ratio of advection to convection in mass flow (Buckley, 2015; Buckley et al., 2015; Buckley et al., 2017). A significant Péclet effect can occur when there is a high velocity of water movement, a long diffusional distance, or a combination of both, such as seen in corn, foxglove and a couple of the oat leaves tested here. The presence of a Péclet effect, as seen in corn, foxglove and oat here, suggests a low resistance pathway from the veins to evaporation sites with a cross-sectional area perpendicular to the direction of flow which is consistent with apoplastic water transport. This may also relate to the variation seen in the mesophyll, such as levels of resistance and distance to evaporation sites which are influenced by stomatal density and may affect the development of gradients (Barbour et al., 2021; Farquhar et al., 2021; Holloway-Phillips et al., 2016). Differences within the mesophyll caused by variation in anatomy may help explain why measurements of transpired vapour only showed enrichment in three out of the four species for the current study.

A recent study by Barbour et al. (2021) investigated how leaf hydraulic design influences the development of gradients and bulk leaf stable oxygen isotope enrichment to determine the relevance of the Péclet effect in leaves. The study examined three different hydraulic designs across a wide range of species, with a few that are comparable to species used in the current study, such as wheat, bean, cotton, and sunflower. Wheat was grouped into hydraulic Design 1, which is described as having weak hydraulic connections with high resistance found between the veins, mesophyll, and epidermal cells; they also usually lack bundle sheath extensions and have unstructured mesophyll tissue. Design 1 leaves such as wheat are expected to have a low velocity for water transport with high resistance pathways between the vascular and mesophyll cells, creating a strong isotope gradient in this high resistance step but preventing the development of gradients in the mesophyll (Figure 3.1). This is consistent with strong increases in enrichment along the leaves and strong carry-forward of the $H_2{}^{18}O$ label in this study (i.e., a strong vascular Péclet effect), but no significant relationship exists between transpiration rate and the leaf water fractional difference.

The findings of Barbour et al. (2021) for bean and cotton support the results of foxglove in the current study, which showed carry-forward of the label and a positive relationship between transpiration rate and the leaf water fractional difference. Bean and cotton were placed under hydraulic Design 2, described as having good hydraulic connections with high resistance in the mesophyll and are expected to have bundle sheath extensions with structured mesophyll. Water transport in Design 2 leaves is dominated by apoplastic flow in the liquid phase, producing a high velocity in areas of low resistance, meaning gradients are likely to form, allowing the occurrence of both vascular and mesophyll Péclet effects (Figure 3.1).

The findings of Barbour et al. (2021) on the hydraulic design of sunflower support the results seen here for sunflower and help to explain why no Péclet effect was seen.

Sunflower was grouped under hydraulic Design 3, where all tissues have good hydraulic connections and indistinct mesophyll. Design 3 leaves generally have a high expression of aquaporins preventing the formation of gradients, and as the mesophyll is well connected, the cross-sectional area for flow increases, producing a slower velocity. These factors result in no mesophyll Péclet effect and no opportunity for a vascular Péclet effect because water within the vascular cells is close to the source water isotope composition (Figure 4.1).

Table 4.1 Summary of evidence for vascular and mesophyll Péclet effects in different species and assumed leaf hydraulic design.

	Evidence of Péclet effects			
Species	**Vascular Péclet effect** **Vapour label**	**Vascular Péclet effect** **Spatial variation**	**Mesophyll Péclet effect** **1-Δ_L/Δ_e**	**Assumed leaf hydraulic design**
Corn	Yes	Yes Song and Barbour., 2016 Gan et al., 2003	No Barbour pers. comm.	Design 1
Oat	Yes not significant	Yes Helliker & Ehleringer 2000 – grasses Kodama et al. 2011 – Triticale	No Barbour et al. 2021 - Wheat	Design 1
Foxglove	Yes	No	Yes	Design 2
Sunflower	No	Yes but increases minor compared to monocots Wang and Yakir 1995	No Barbour et al. 2021	Design 3

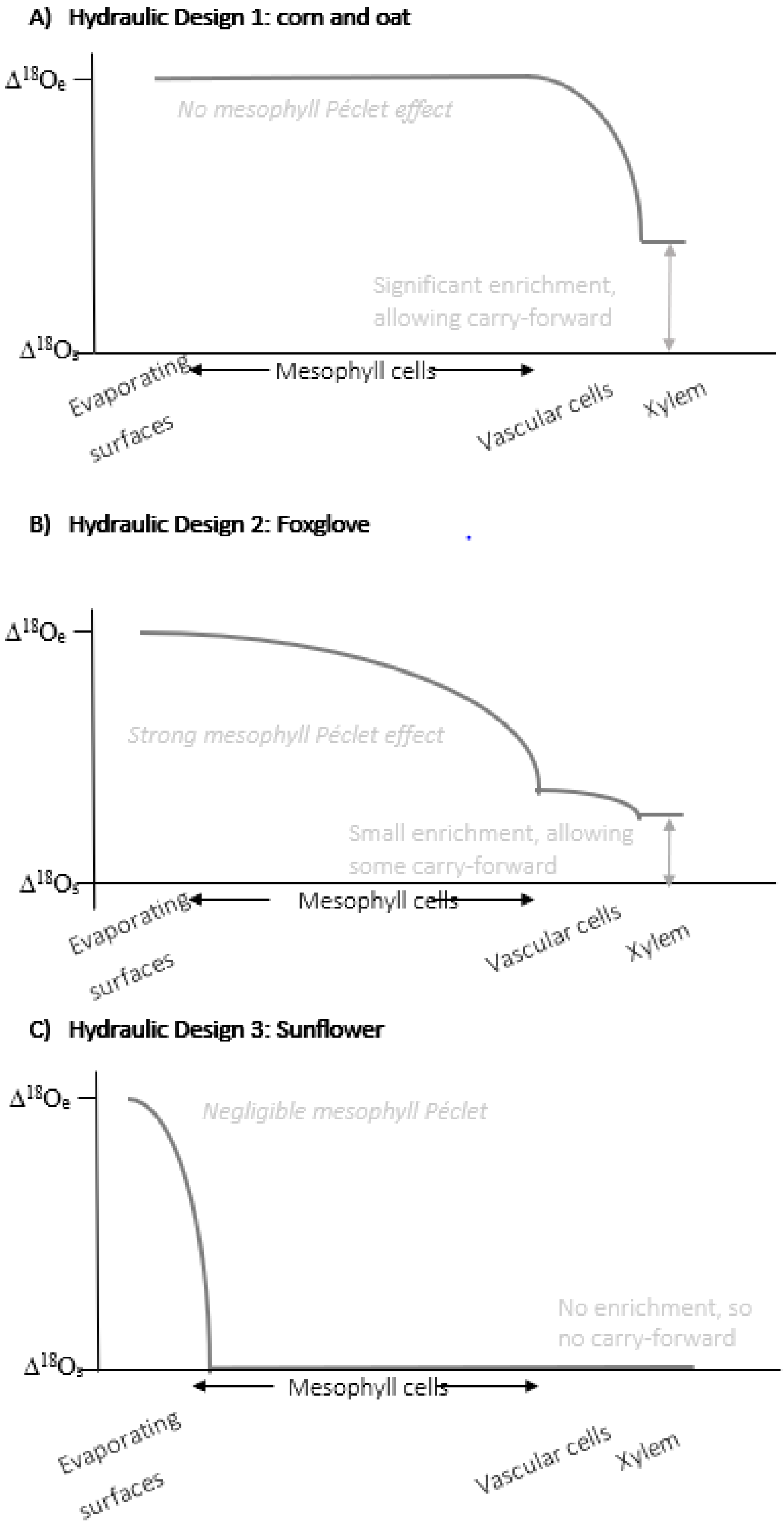

Figure 4.1 Hydraulic design and associated Péclet Effects for all species

4.6 Conclusions

This study set out to address the question of how relevant the Péclet effect is to patterns of leaf water isotopic enrichment in monocot and dicot species. The first experiment aimed to test for evidence of a vascular Péclet effect by examining different levels of enrichment observed in measurements of transpired vapour and calculated leaf water in monocot and dicot leaves. Experiments Two and Three aimed to quantify the spatial variation of $\delta^{18}O$ in leaf water of foxglove (a vascular Péclet effect) and to compare $H_2{}^{18}O$ enrichment in leaf water with the Craig-Gordon model predicted enrichment to determine the relevance of a mesophyll Péclet effect in foxglove. These experiments showed that a vascular Péclet effect is relevant to leaf water isotopic enrichment in monocots, and some dicot species, because corn, oat and foxglove displayed changes in $\delta^{18}O$, while sunflower did not. The relevance of the mesophyll Péclet effect in foxglove was supported further by a significant positive relationship between transpiration rates and fractional difference. Spatial variation of leaf water in foxglove did not show any evidence for the relevance of a significant vascular Péclet effect but did highlight important differences in hydraulic design between species and types of leaves. This study contributes to our understanding of how the different Péclet effects influence isotopic enrichment of leaf water and transpired vapour and how differences in fluxes and leaf hydraulic design affect which enrichment model is relevant and the extent to which it occurs. The experiments shown here are the first to use vapour isotope labelling to investigate leaf hydraulic pathways, revealing a valuable tool for future research. A limitation of this study was the number of leaf samples used for examining spatial variation in foxglove leaves and for comparing transpiration rates to fractional differences. Another limitation was the number of labelled replicates used for measuring transpired vapour and calculating leaf water enrichment as a mistake such as what happened with oat can easily cause unreliable data and skew results. Further research should be carried out to better understand isotopic enrichment in transpired vapour and the implications for leaf hydraulic design across a range of species.

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Appendix

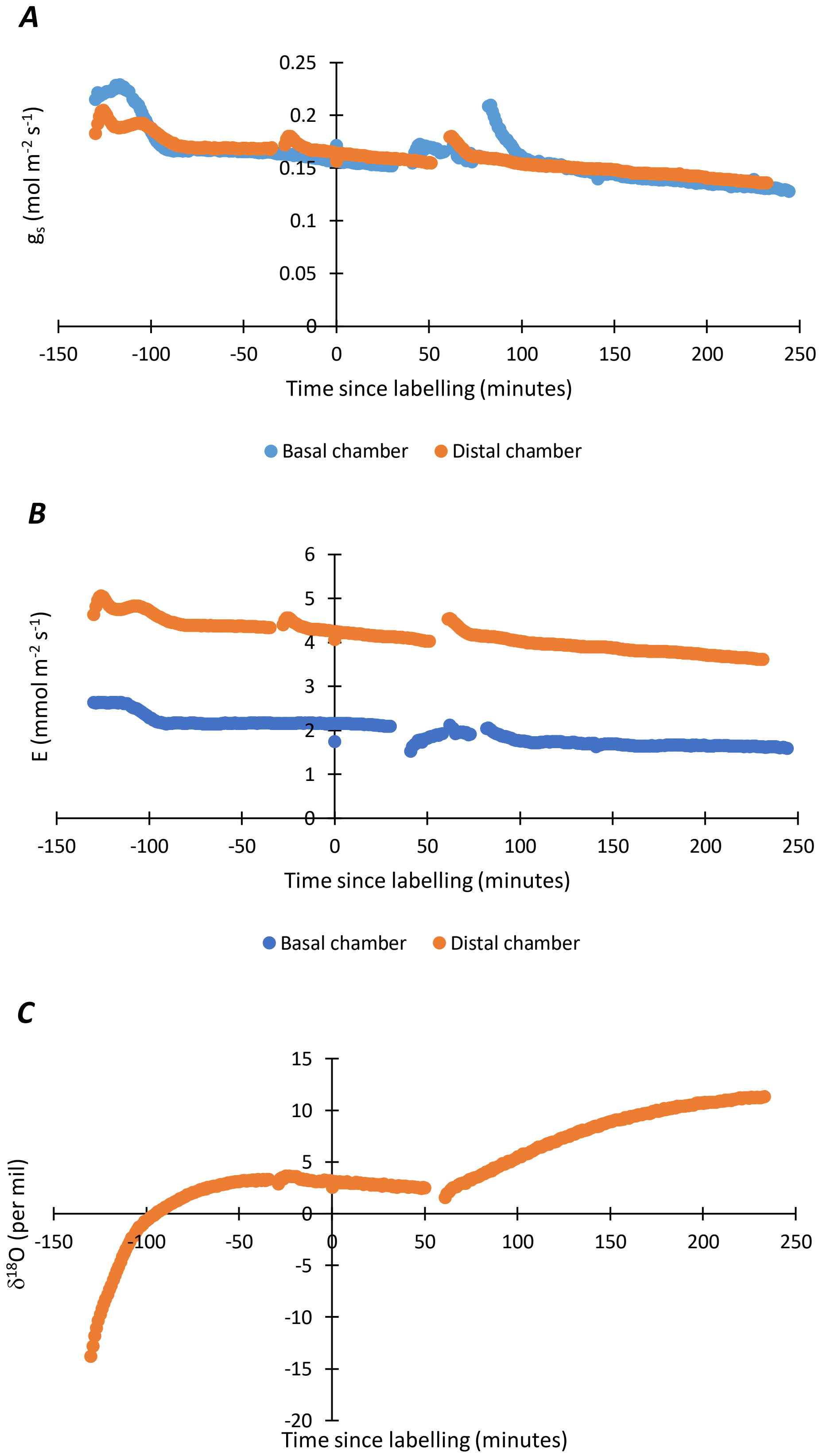


Figure A1. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 1 (label).

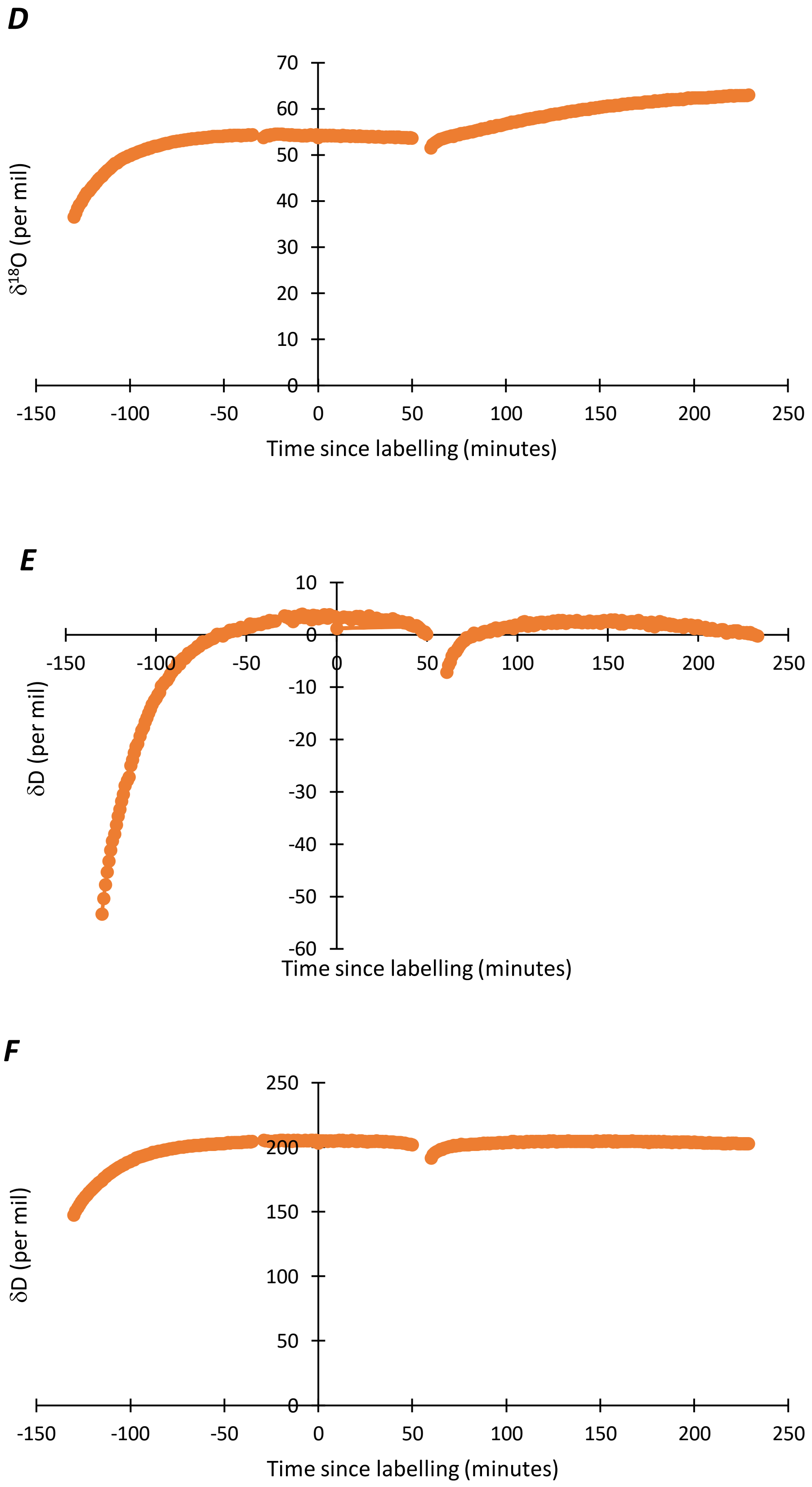


Figure A2. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 1 (label).

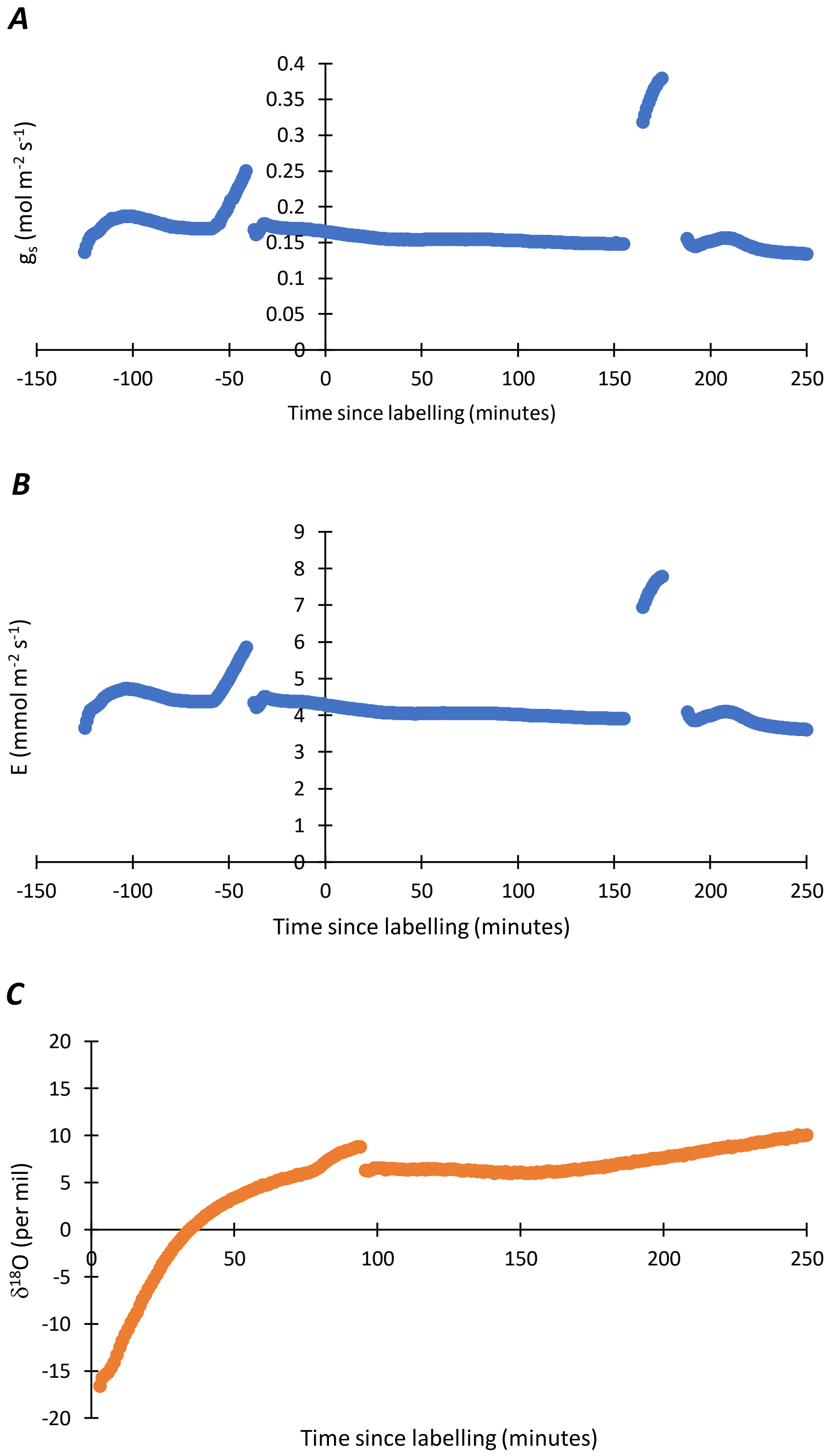


Figure A3. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 3 (label).

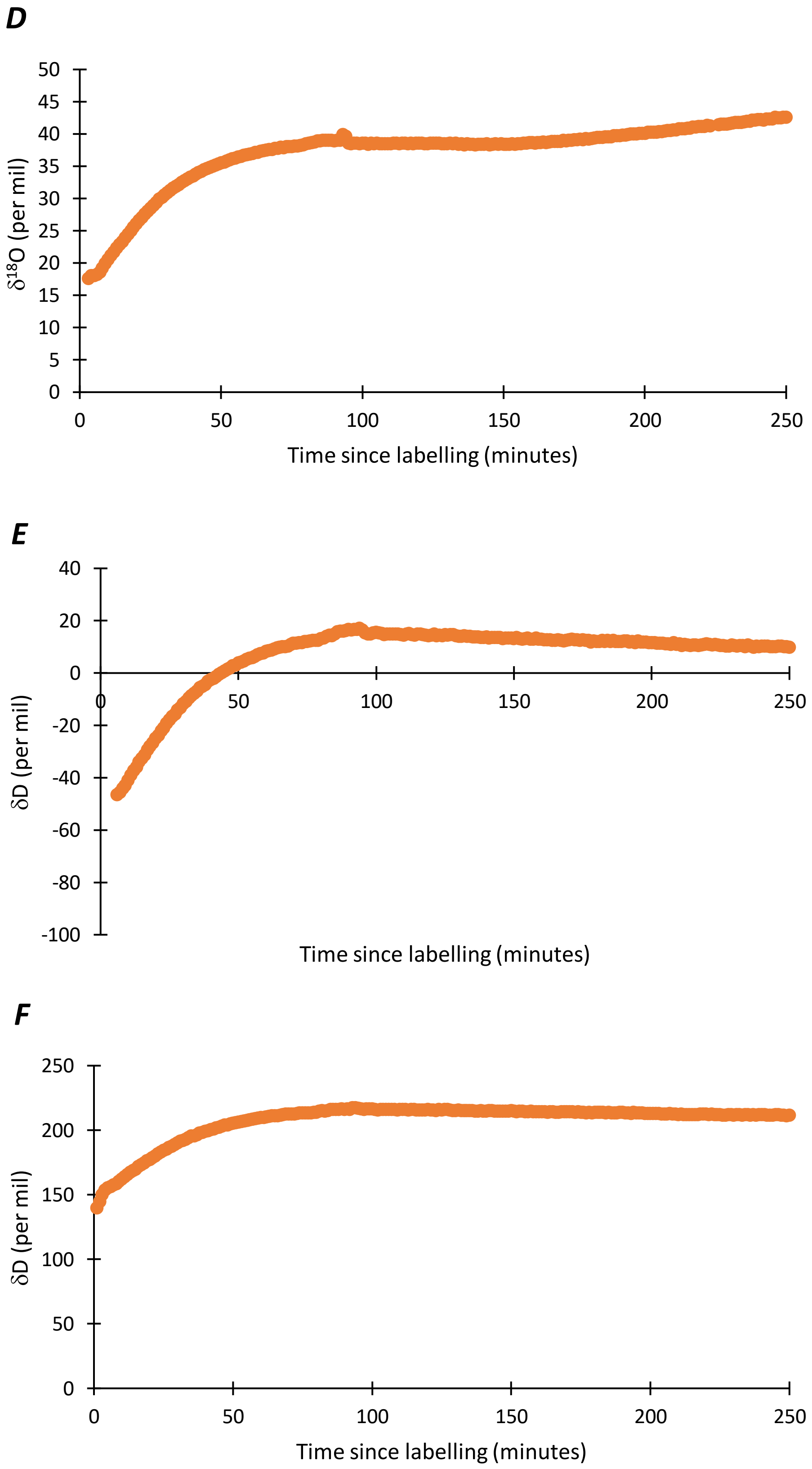


Figure A4. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 3 (label).

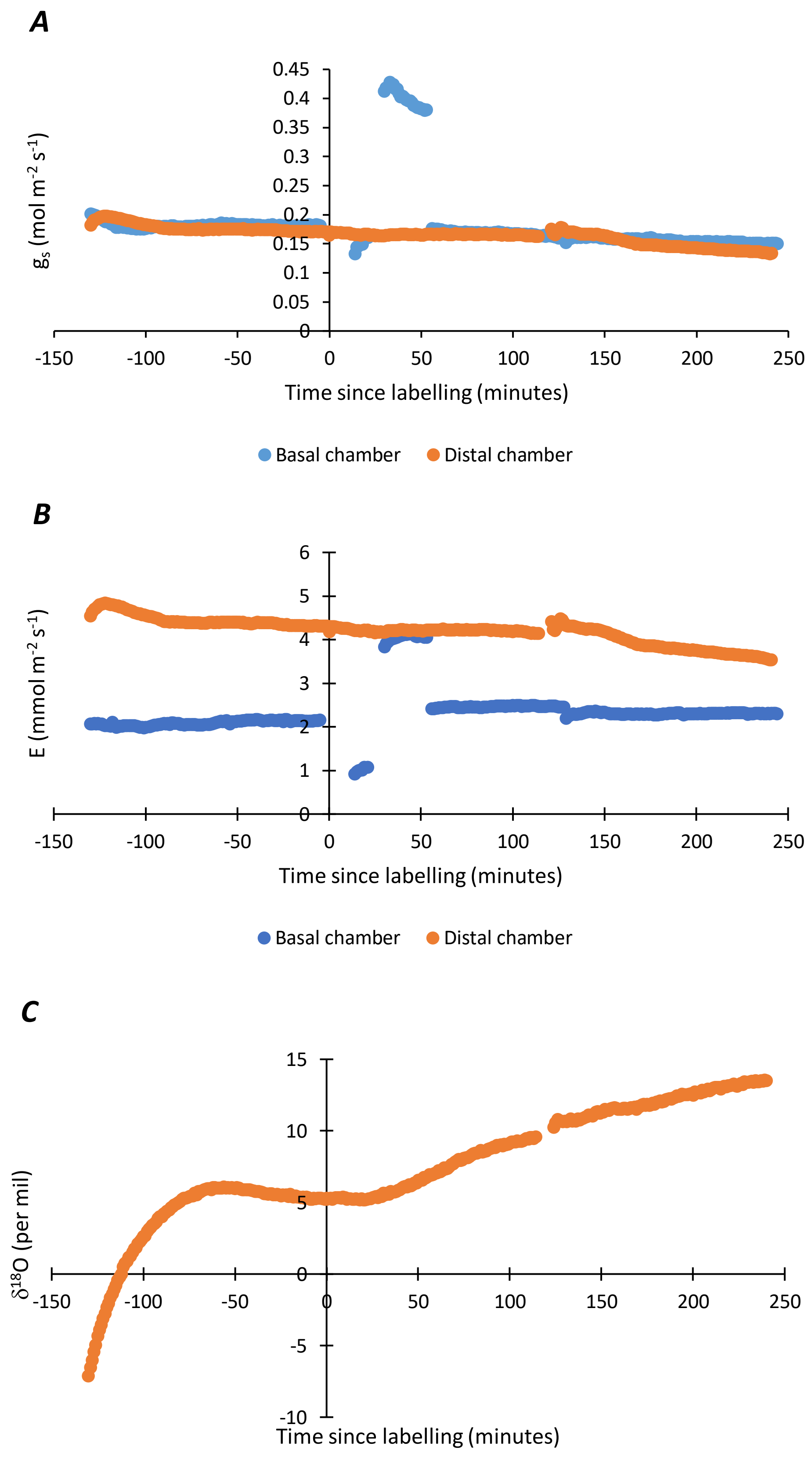


Figure A5. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 8 (label).

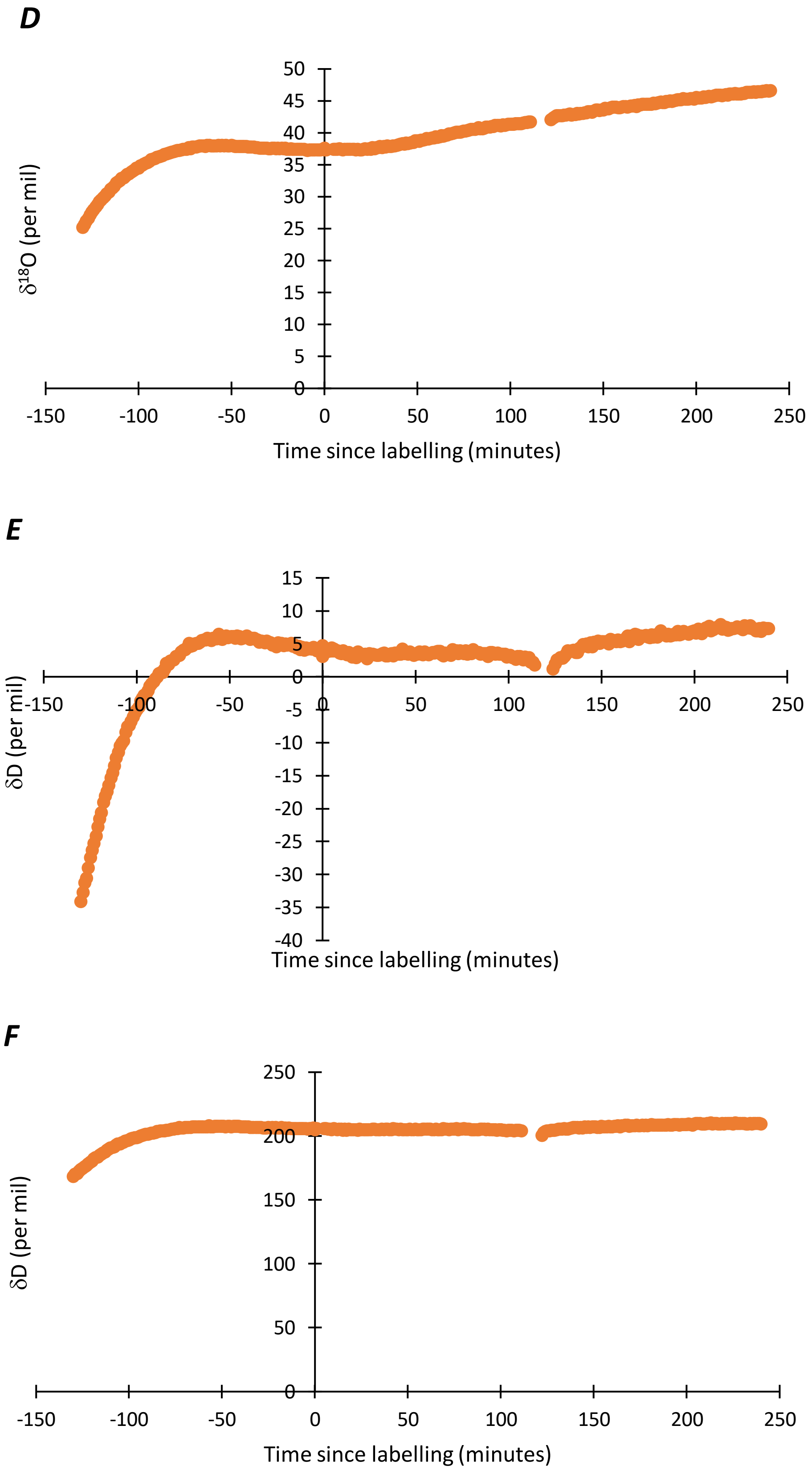


Figure A6. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 8 (label).

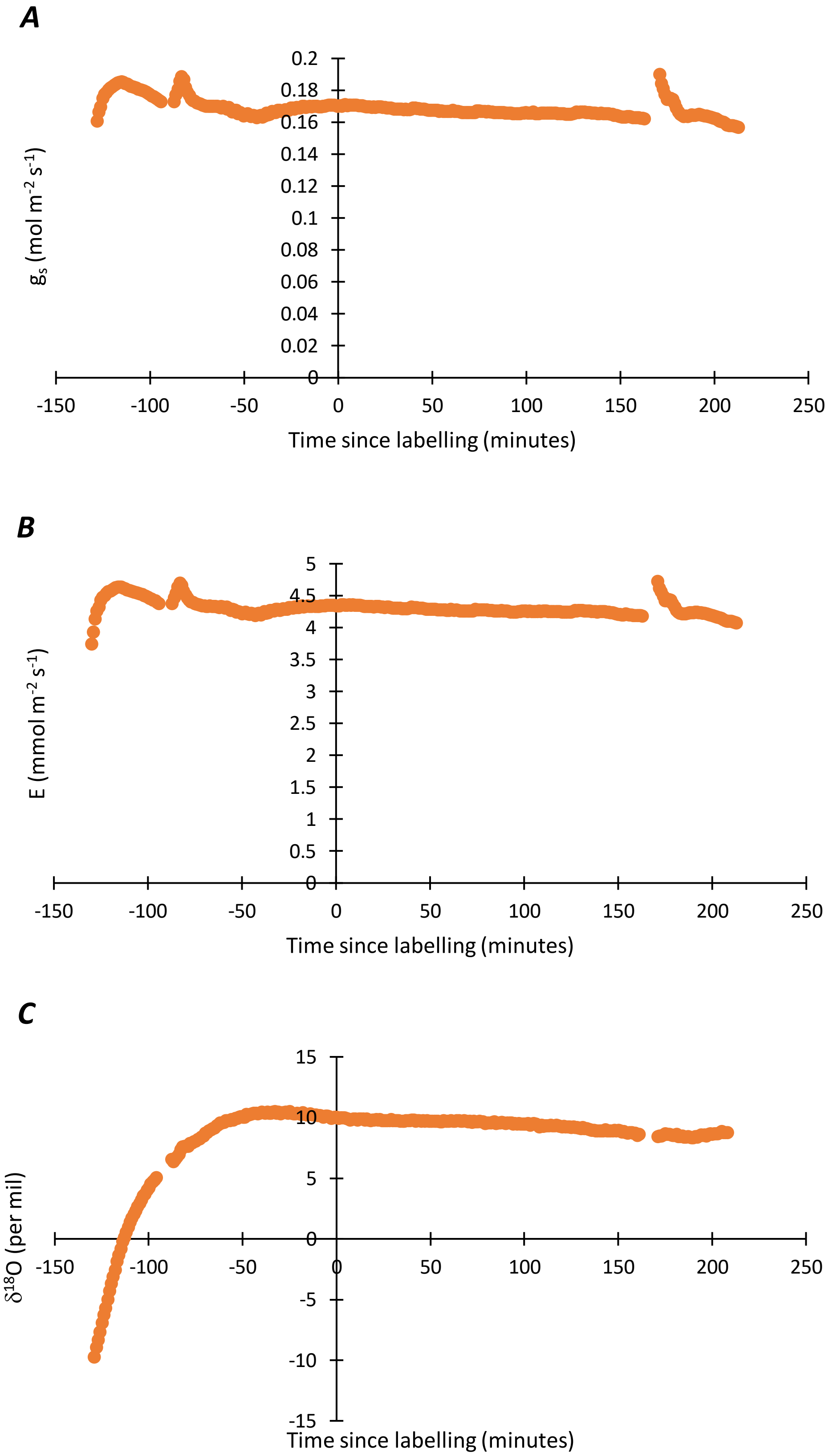


Figure A7. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 4 (control).

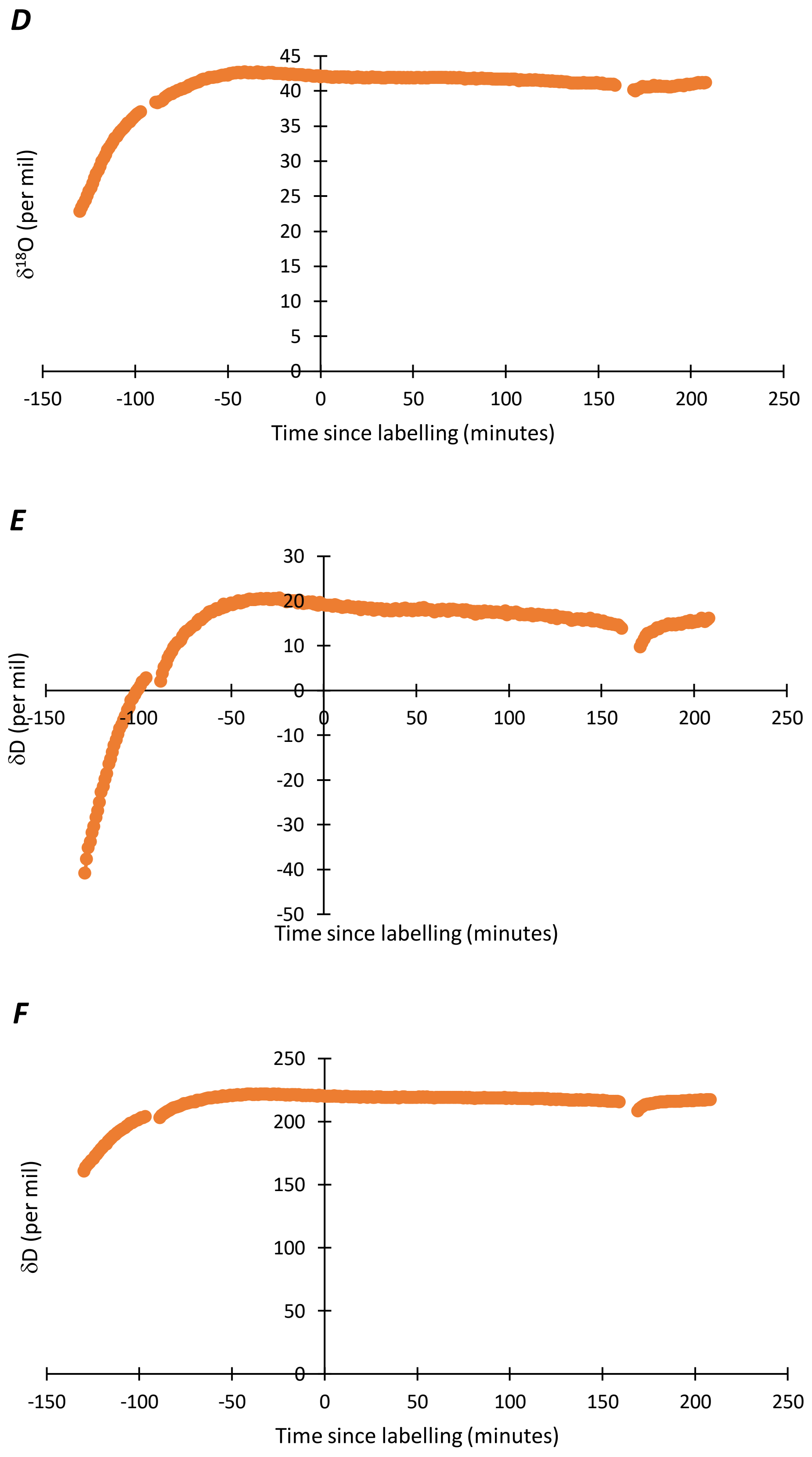


Figure A8. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 4 (control).

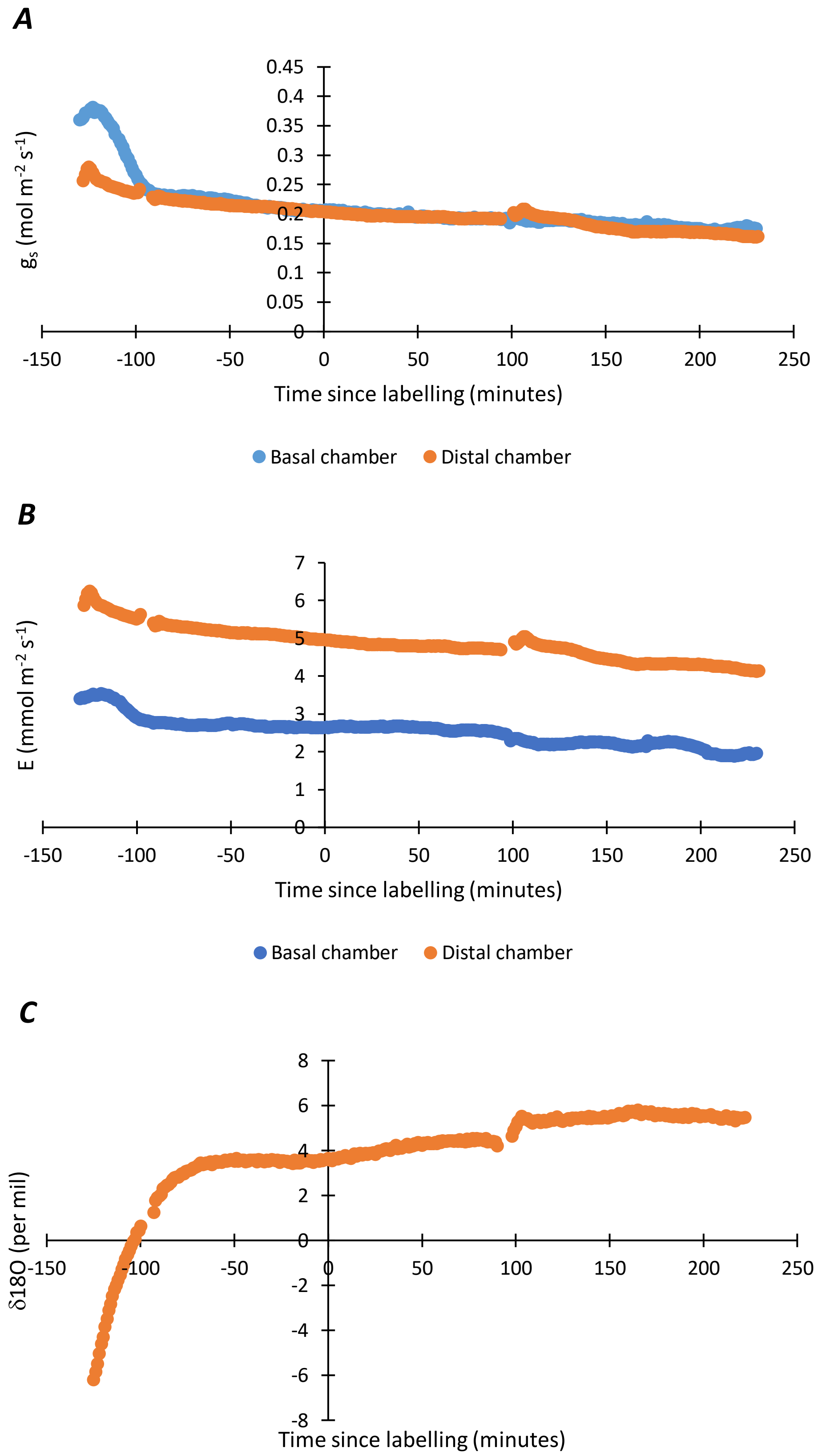


Figure A9. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 7 (control).

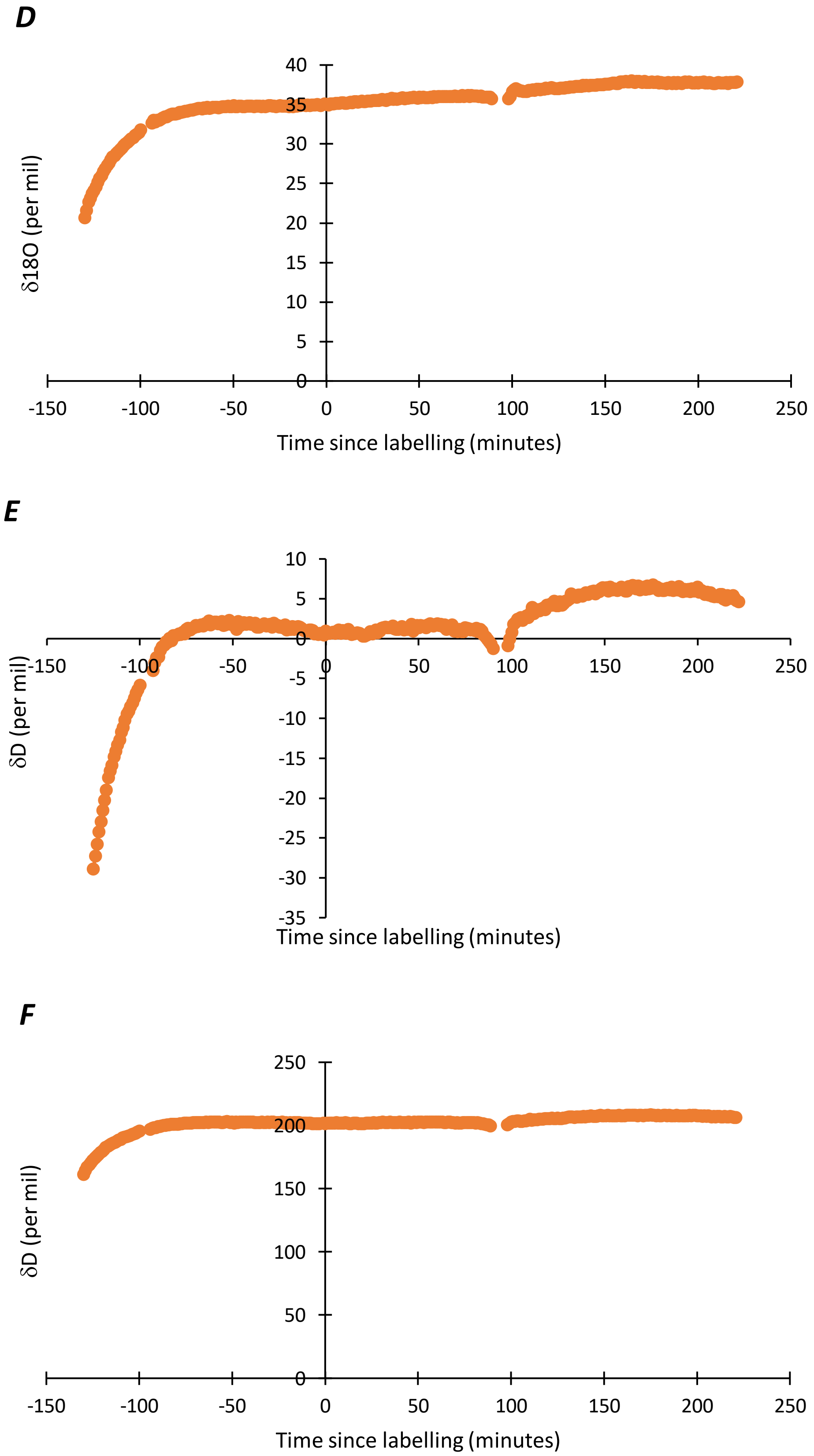


Figure A10. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 7 (control).

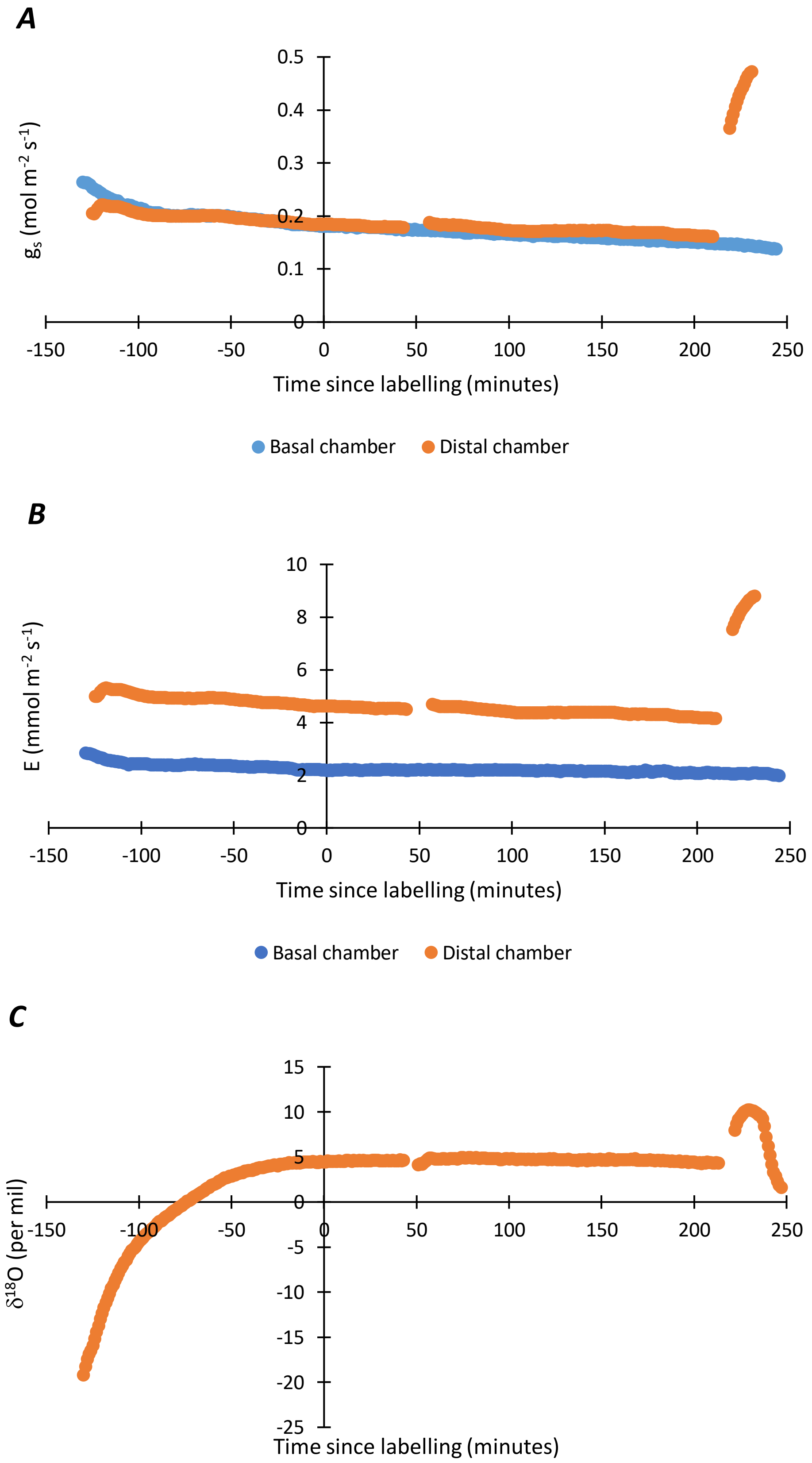


Figure A11. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Corn plant 6 (control).

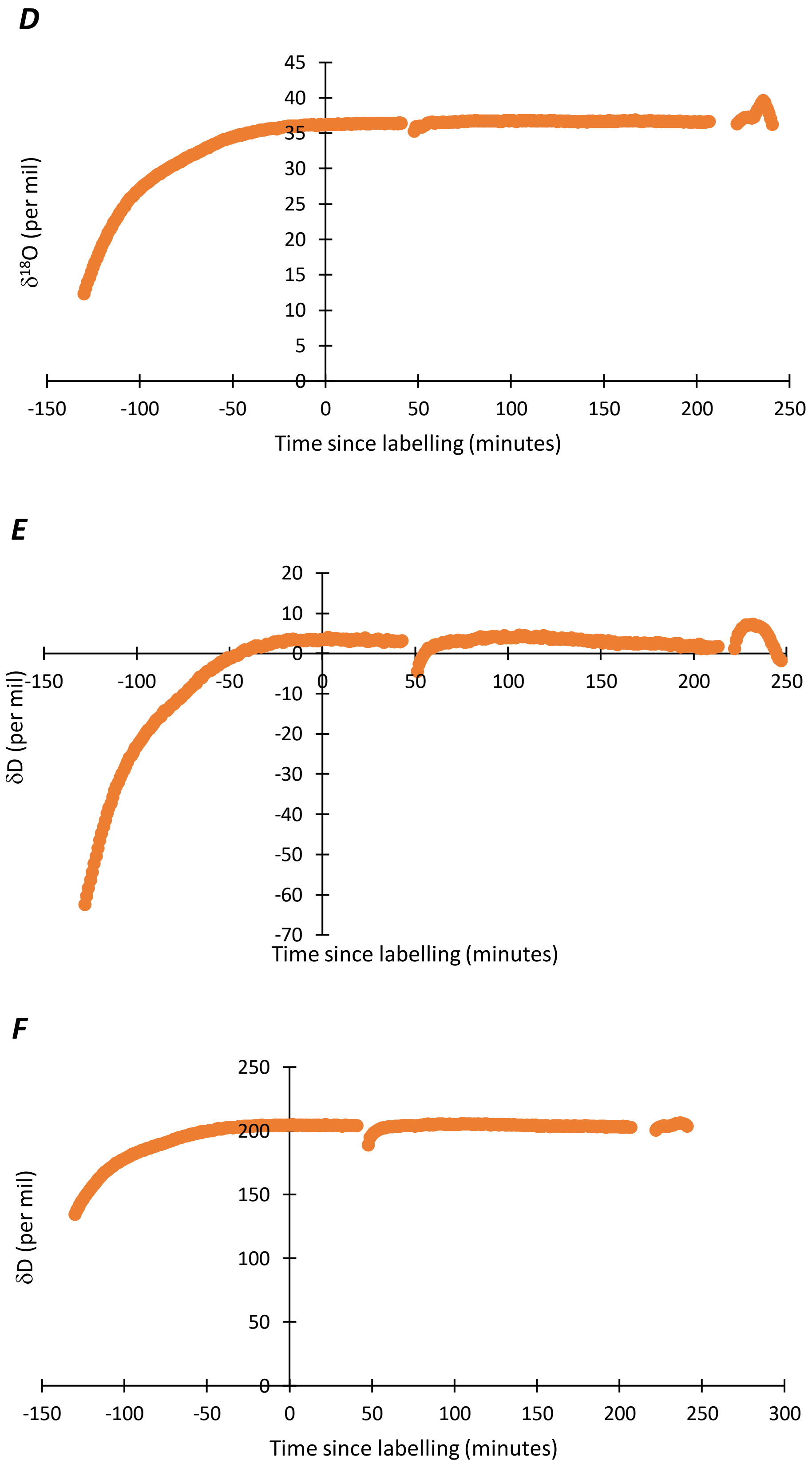


Figure A12. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 6 (control).

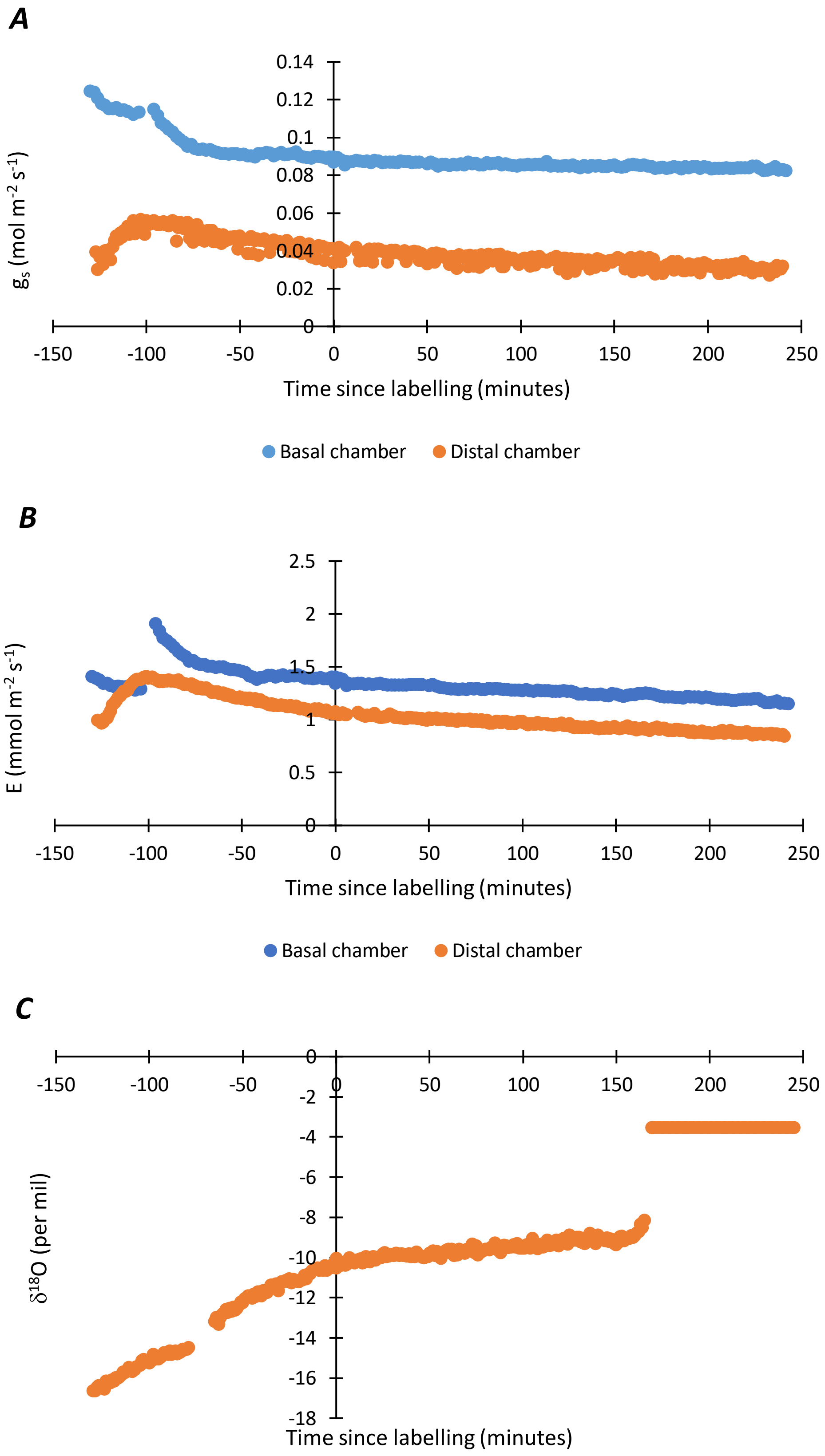


Figure A13. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Foxglove plant 3 (label).

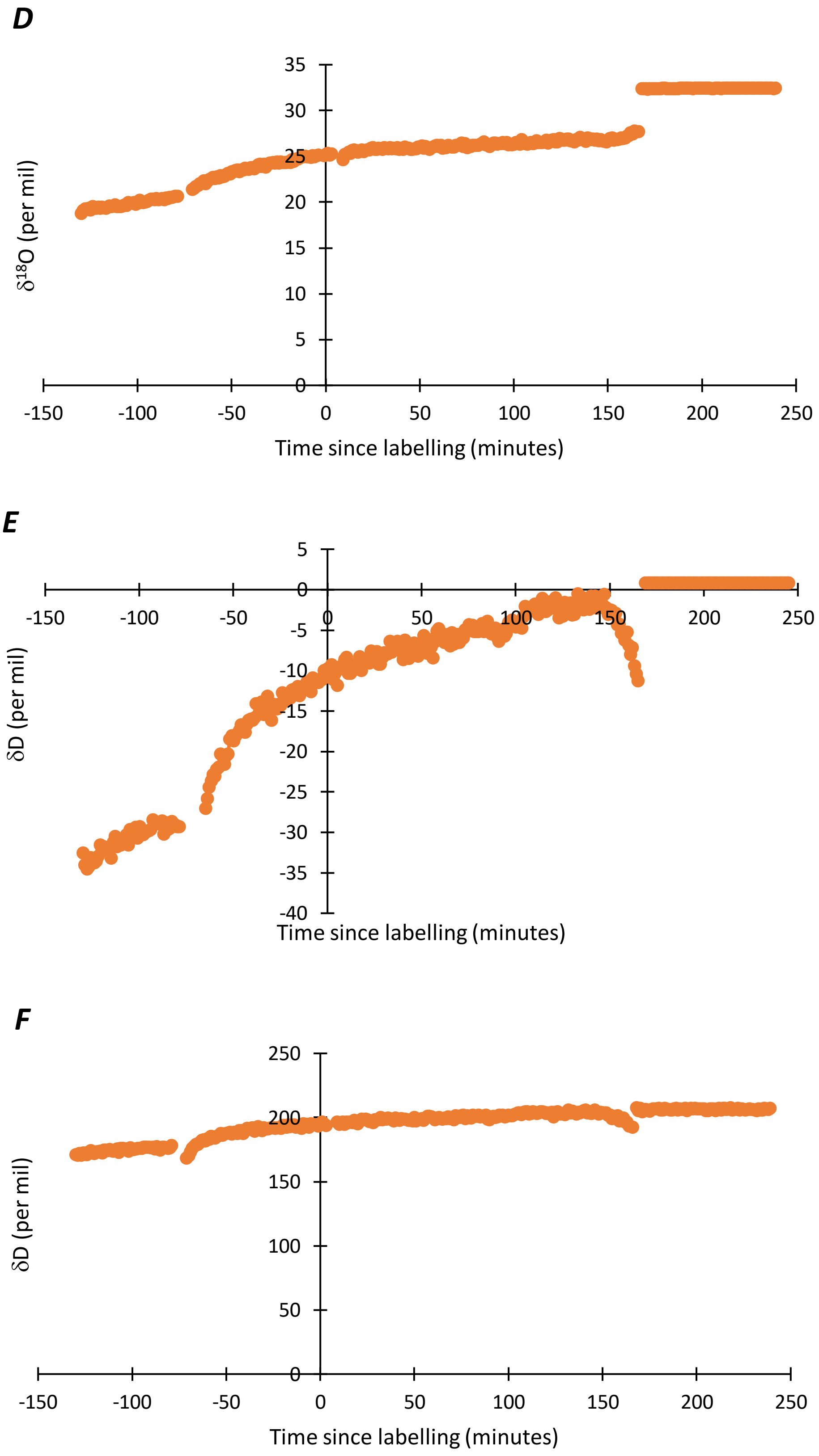


Figure A14. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Foxglove plant 3 (label).

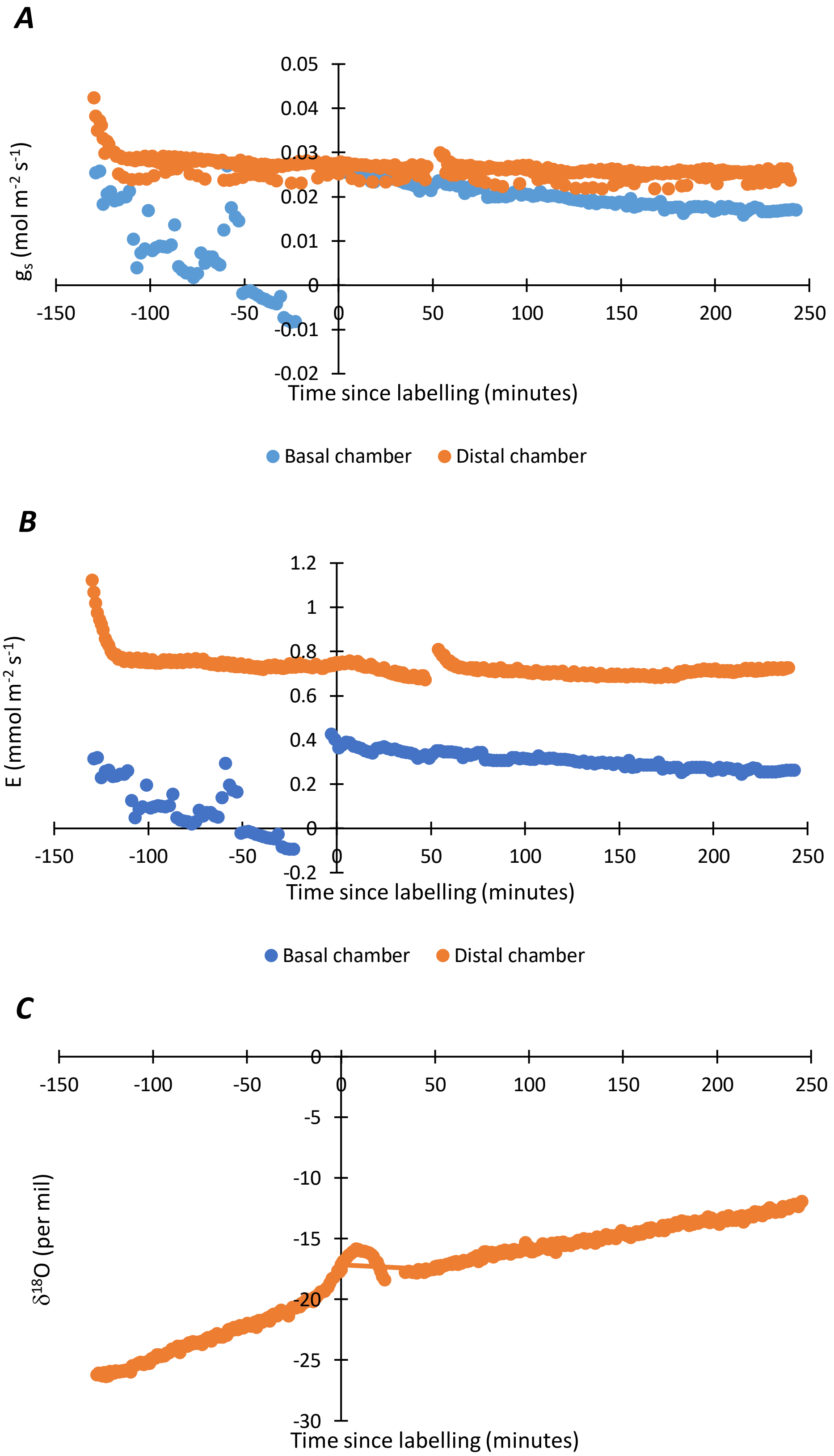


Figure A15. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Foxglove plant 5 (label).

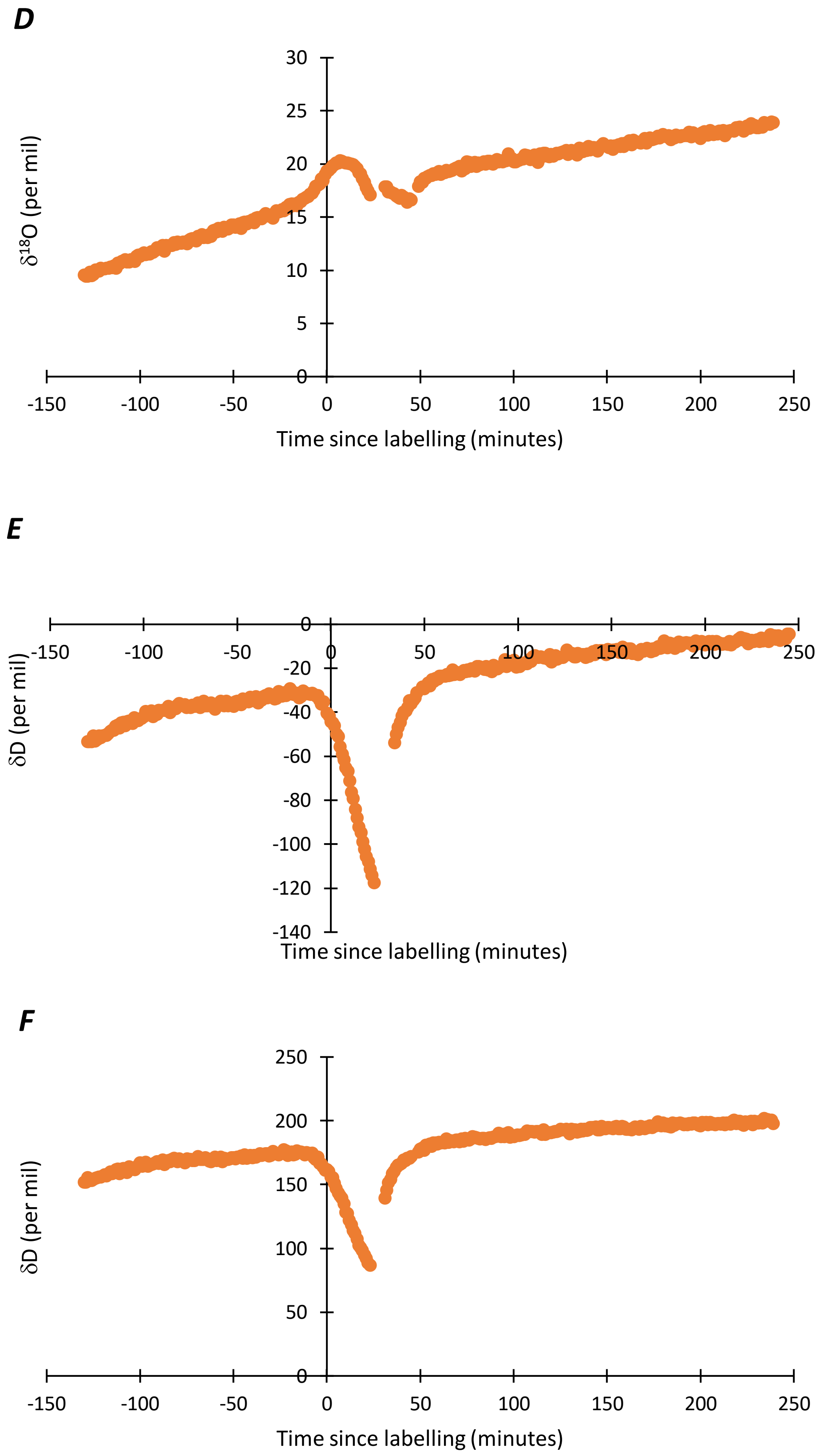


Figure A16. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Foxglove plant 5 (label).

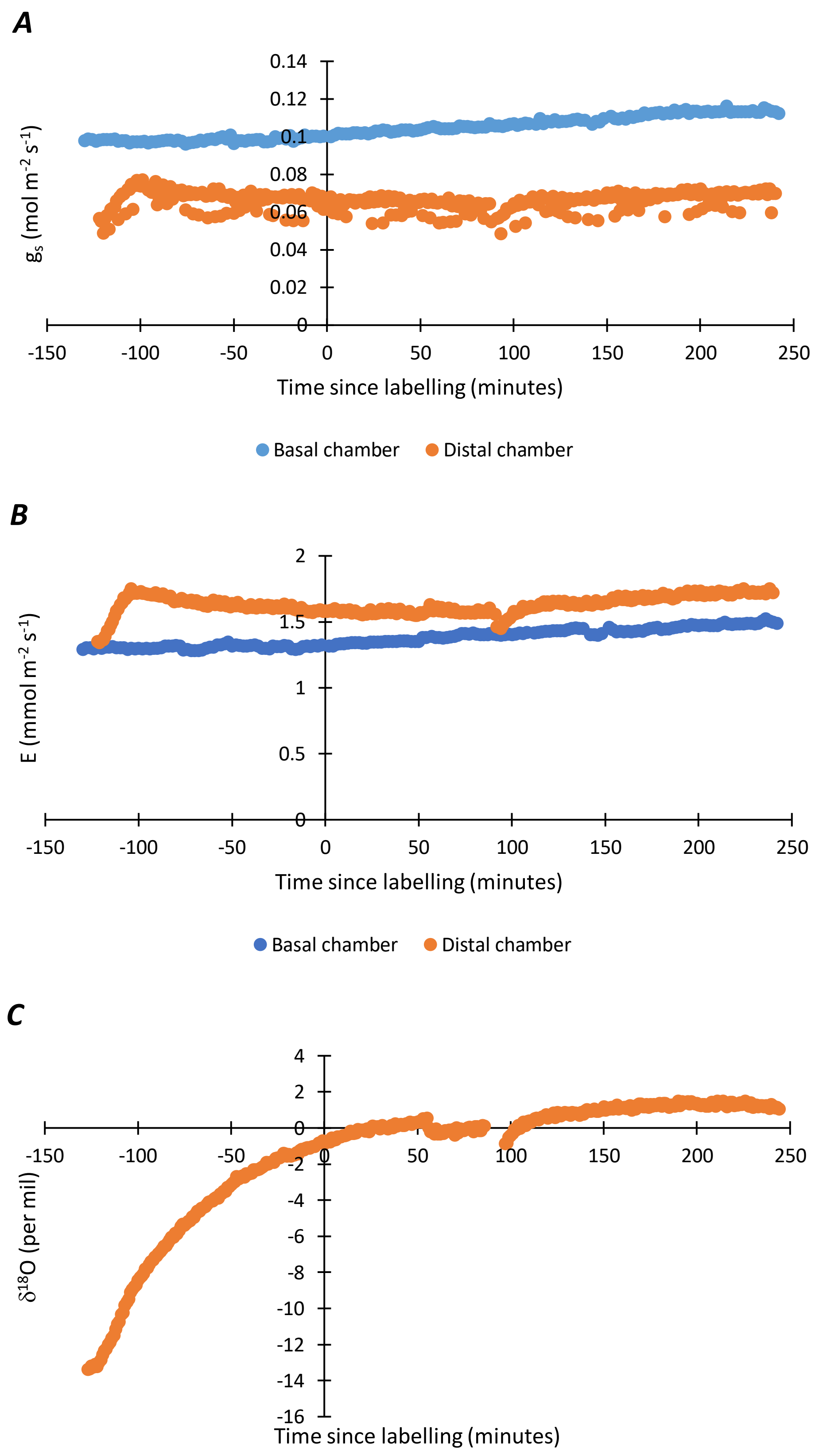


Figure A17. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Foxglove plant 2 (control).

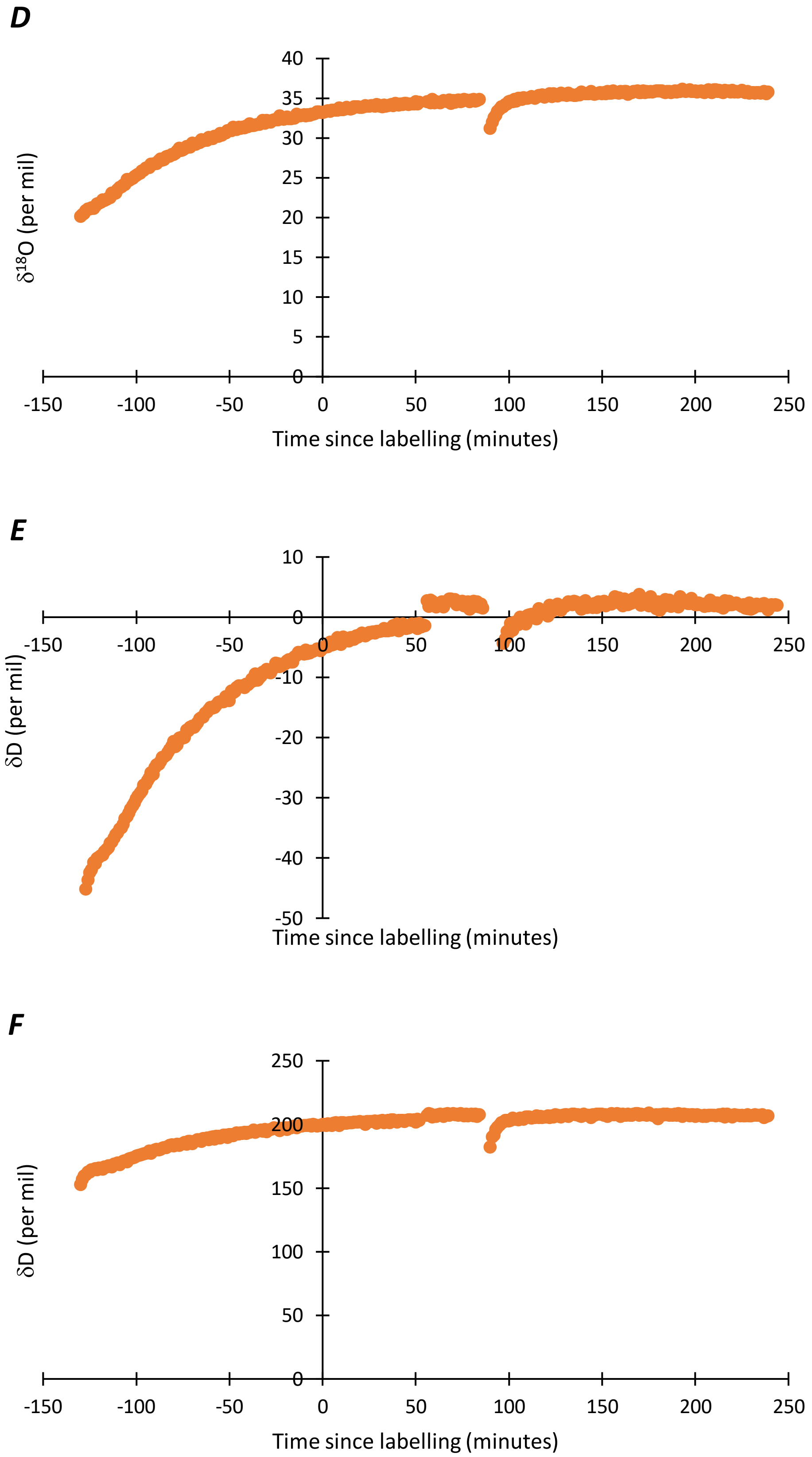


Figure A18. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Foxglove plant 2 (control).

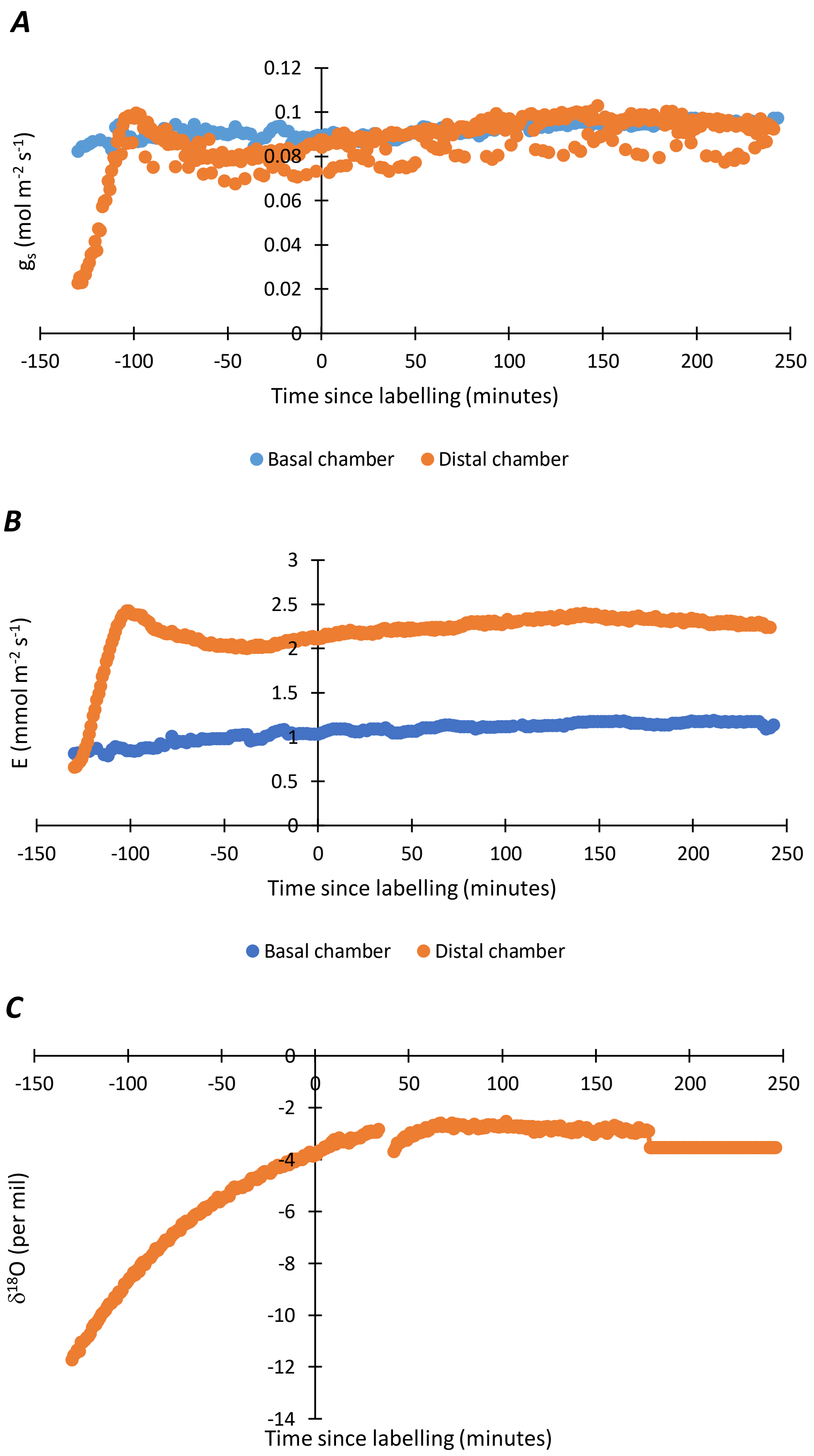


Figure A19. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Foxglove plant 6 (control).

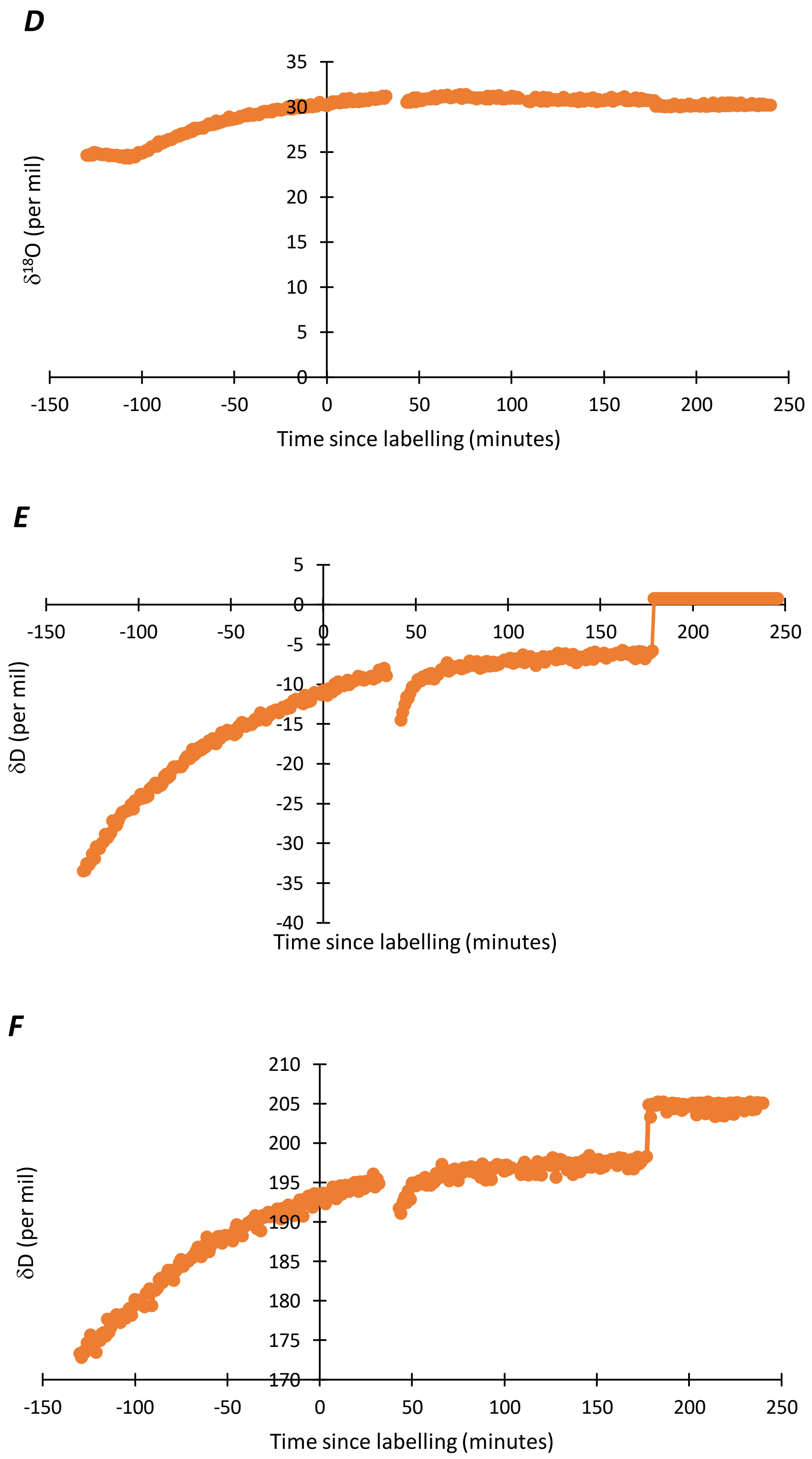


Figure A20. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Foxglove plant 6 (control).

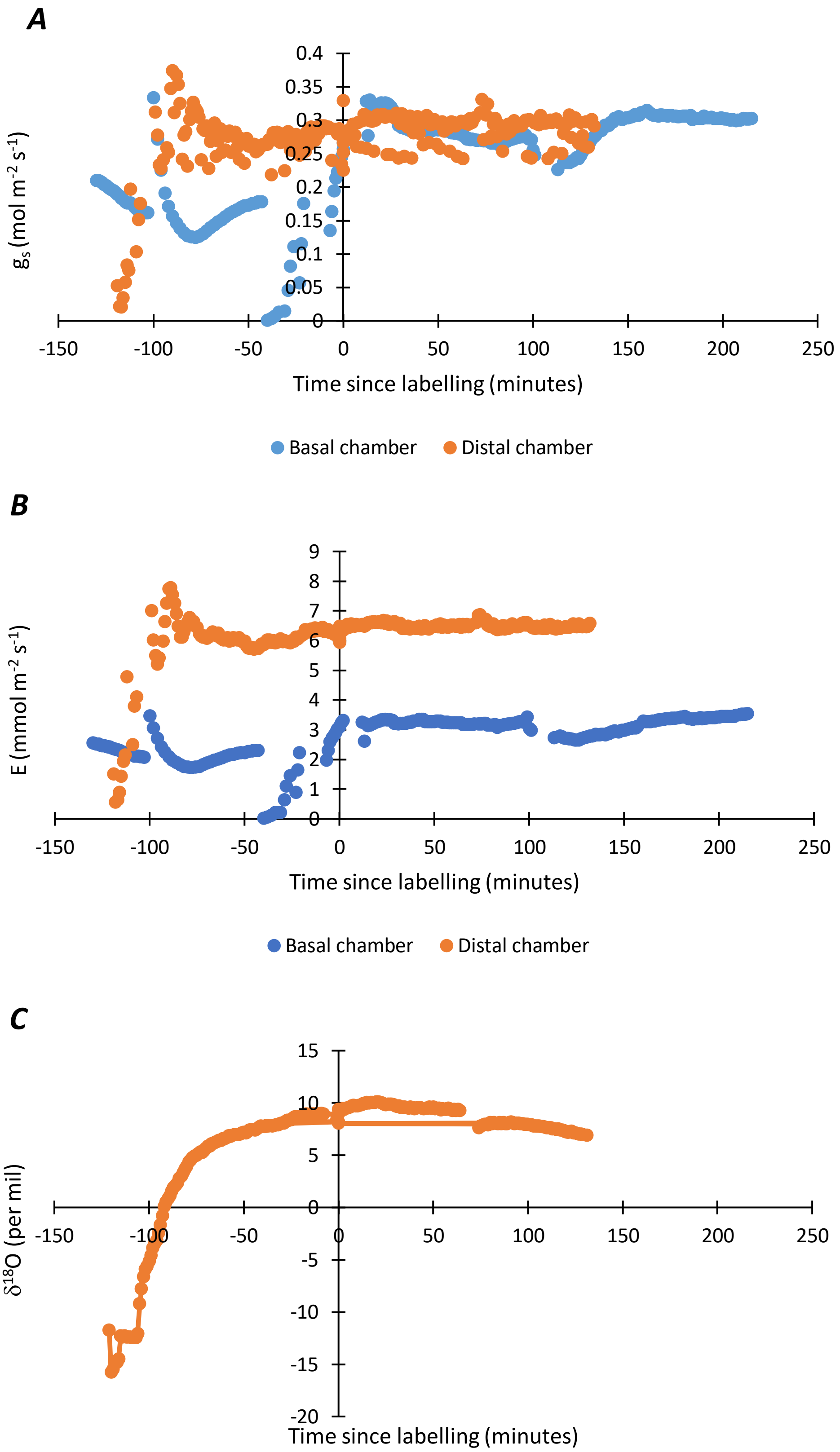


Figure A21. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 1 (label).

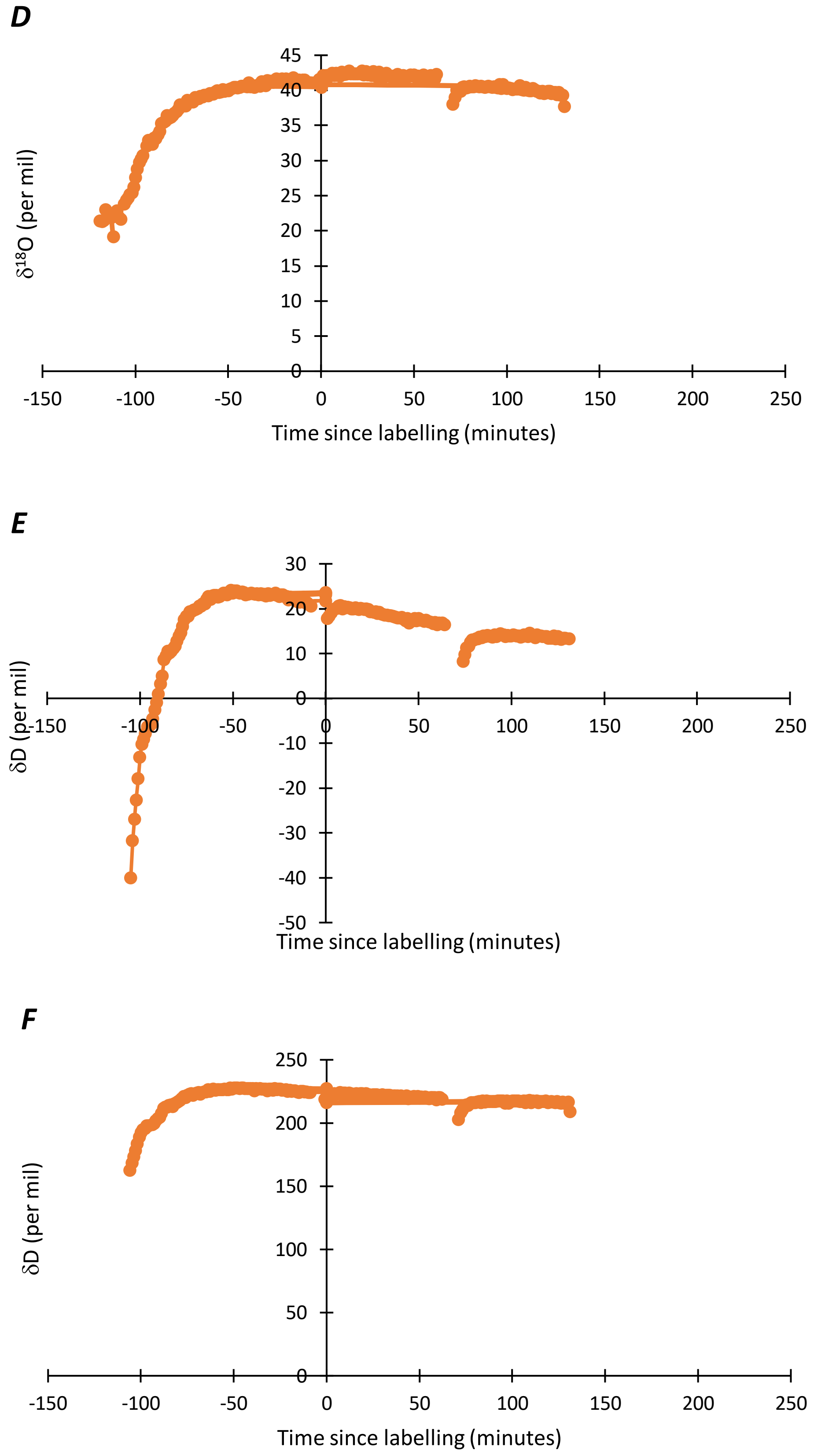


Figure A22. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 1 (label).

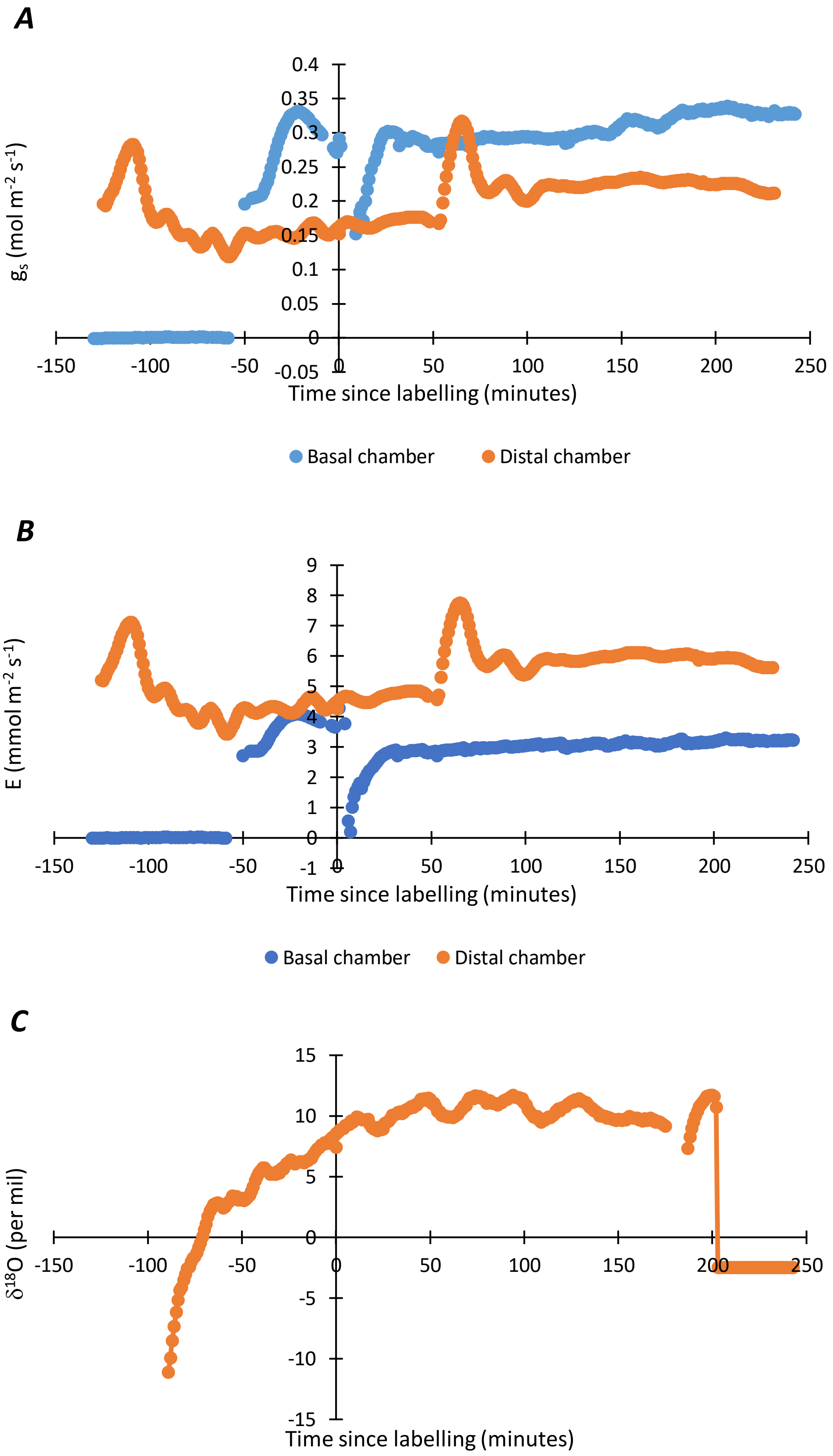


Figure A23. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 3 (label).

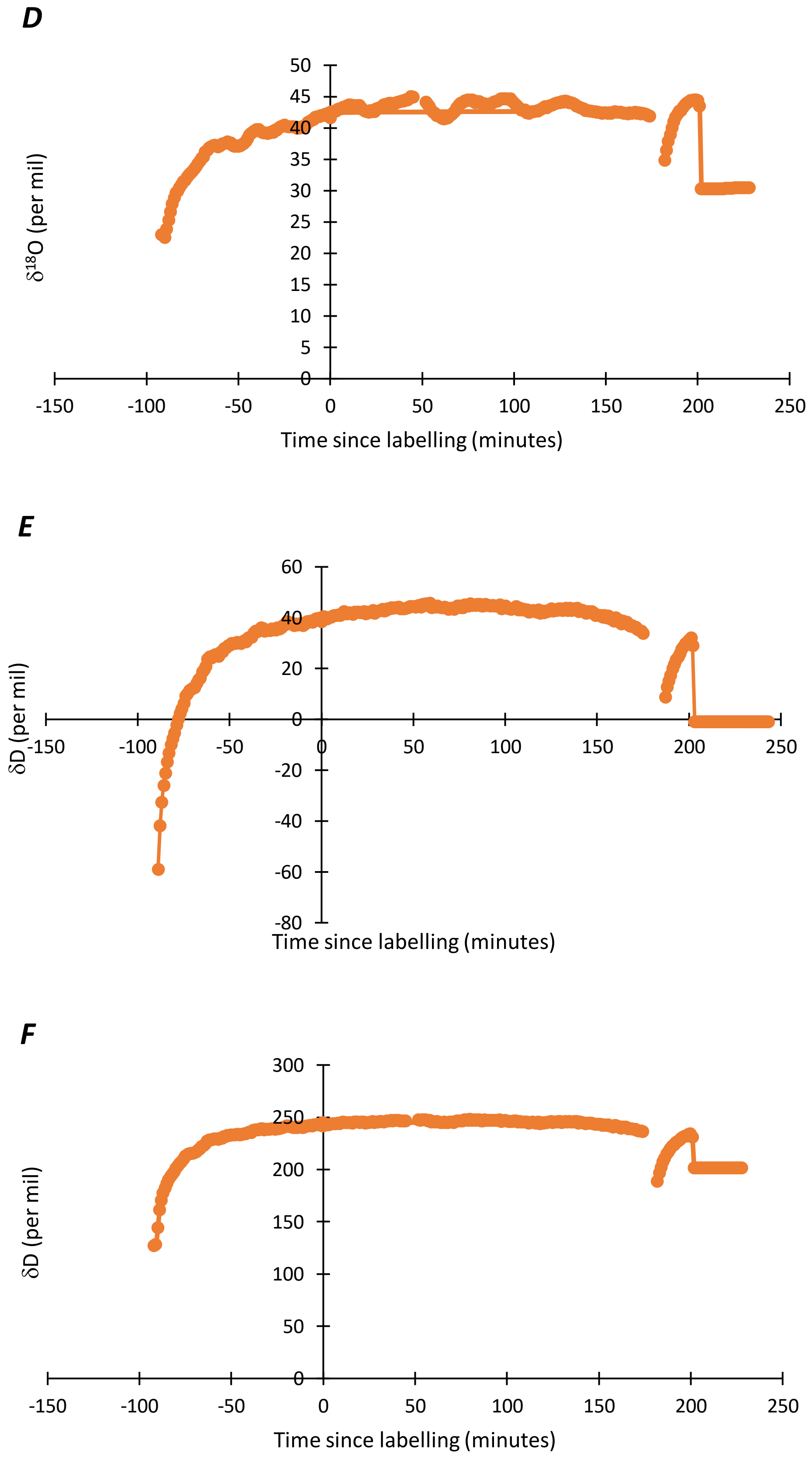


Figure A24. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 3 (label).

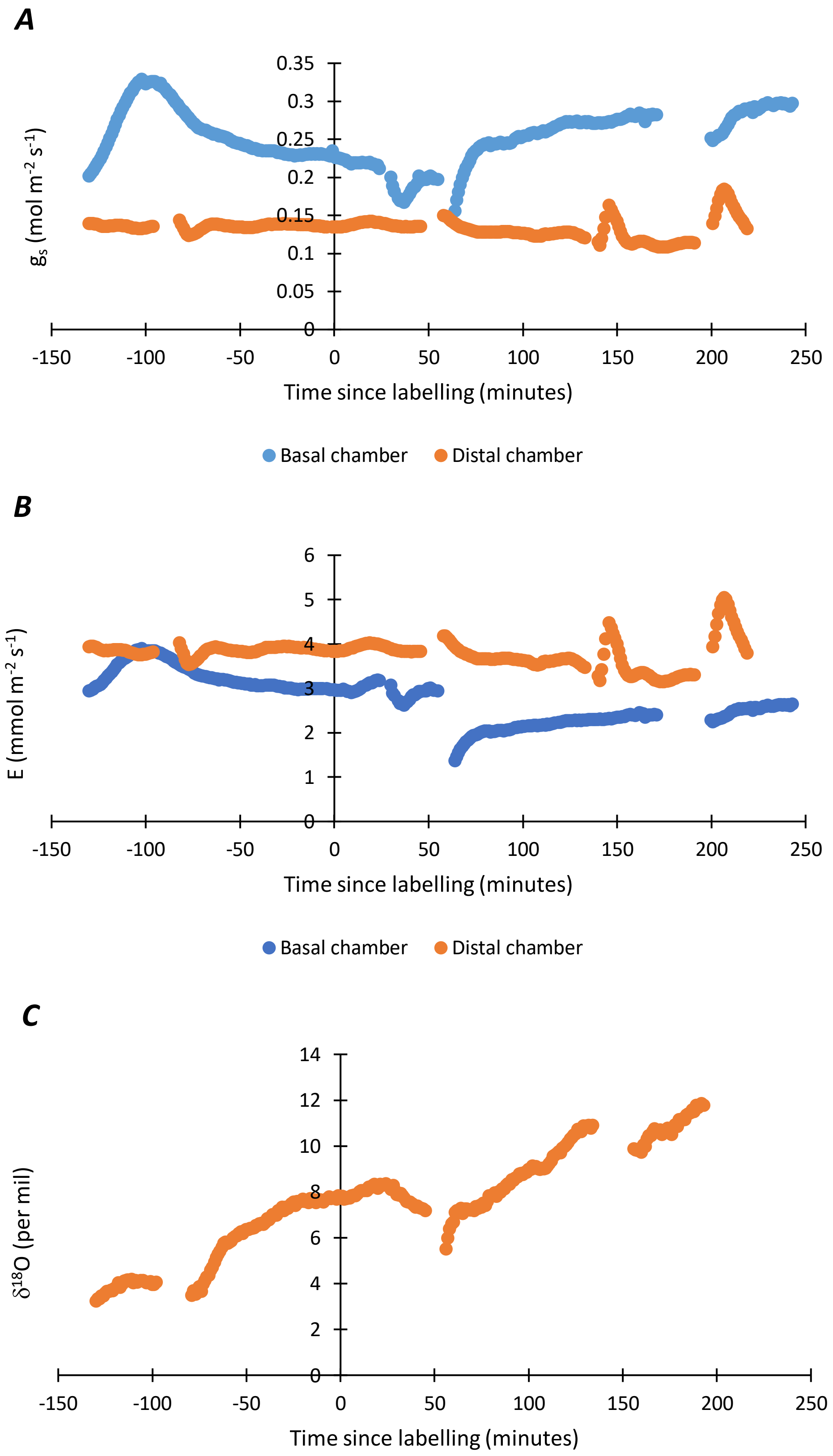


Figure A25. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 4 (label).

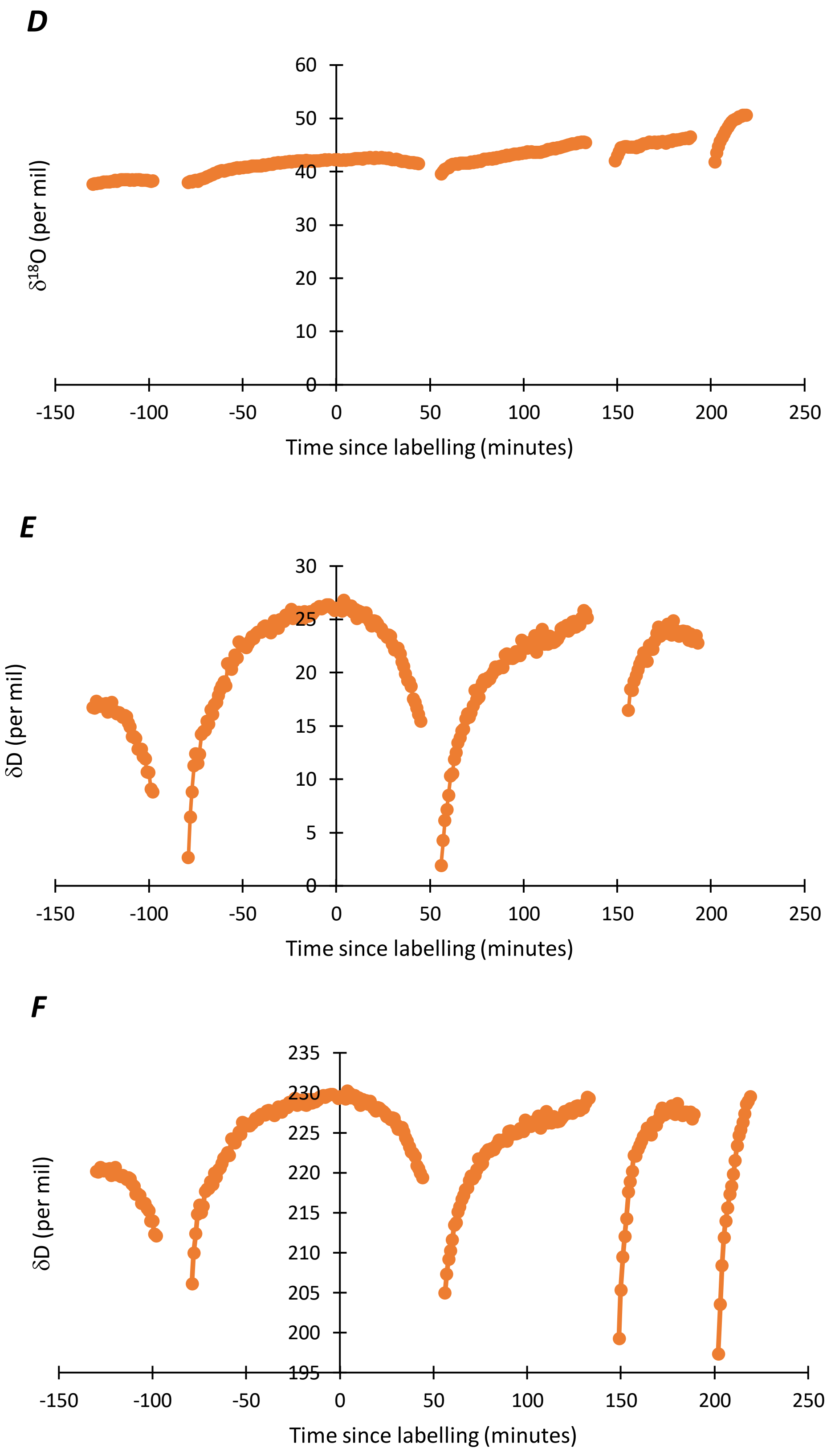


Figure A26. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 4 (label).

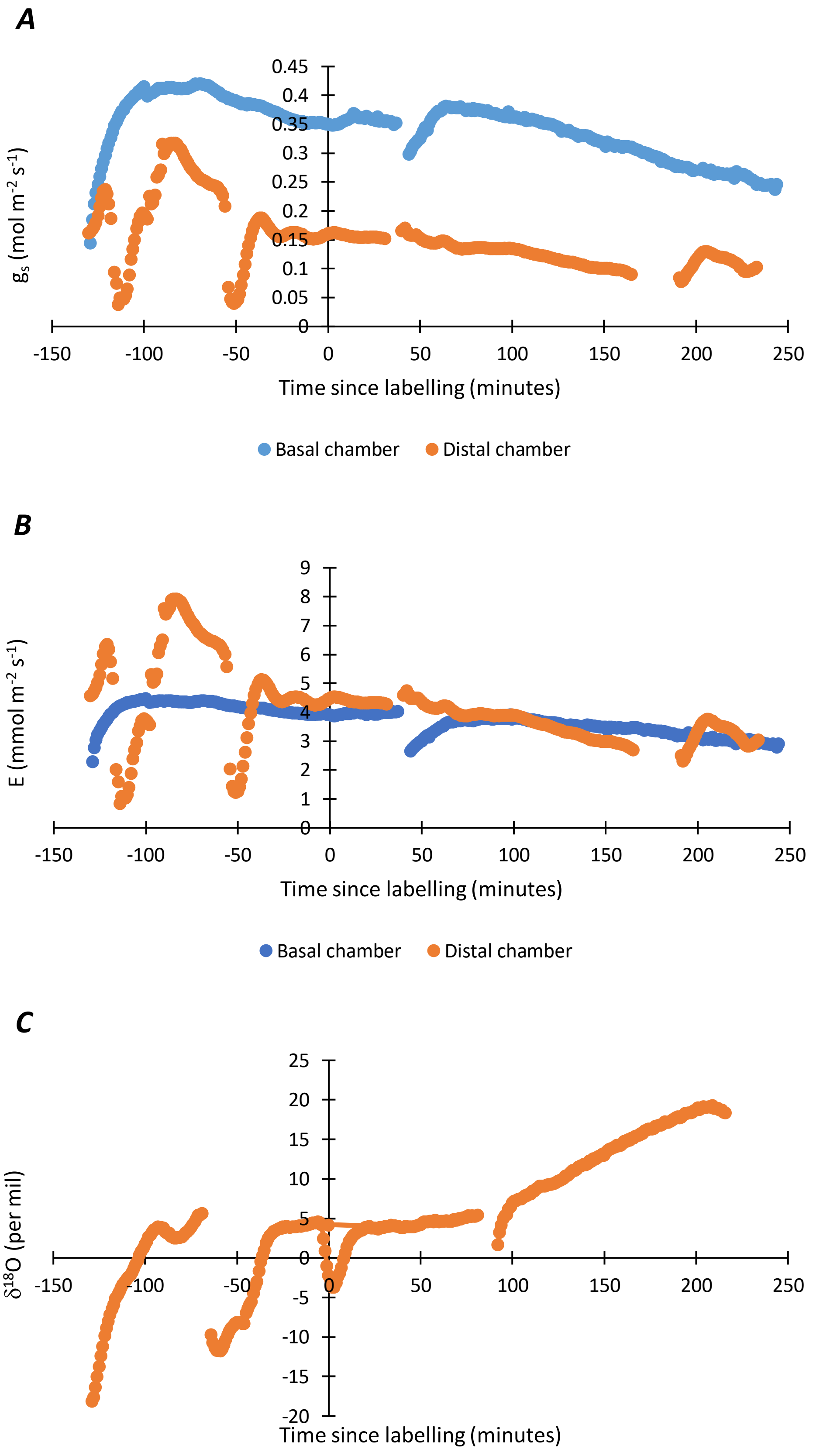
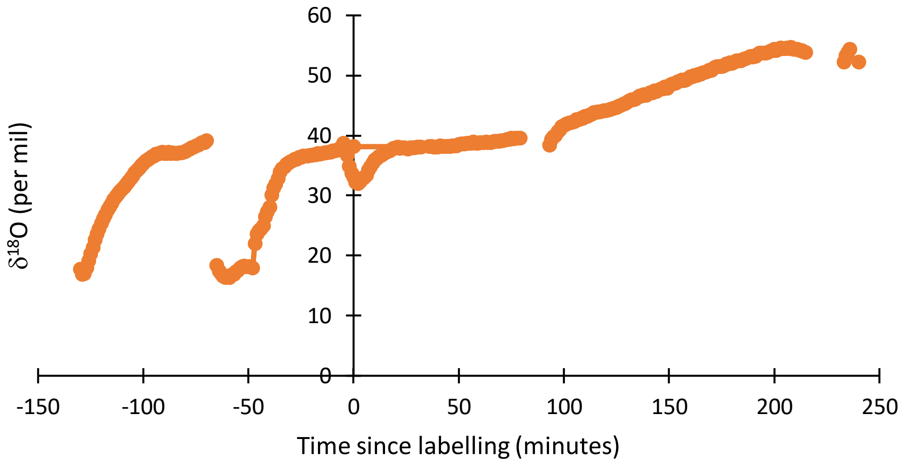


Figure A27. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 5 (control).

D



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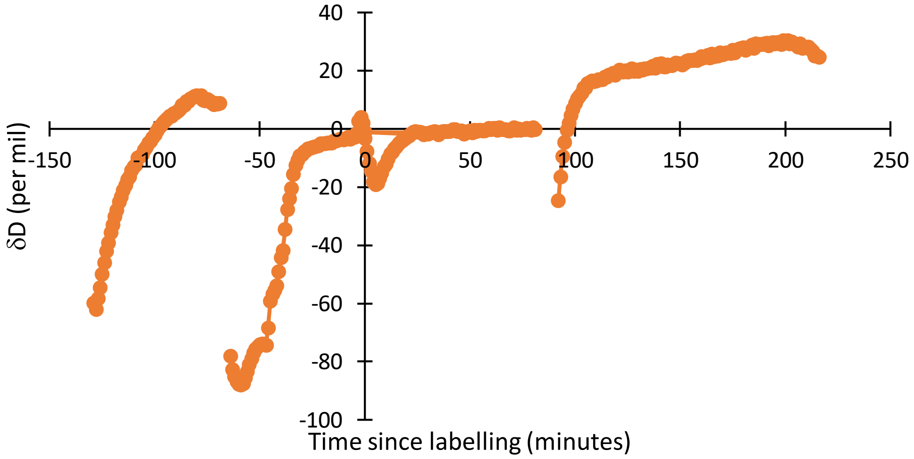

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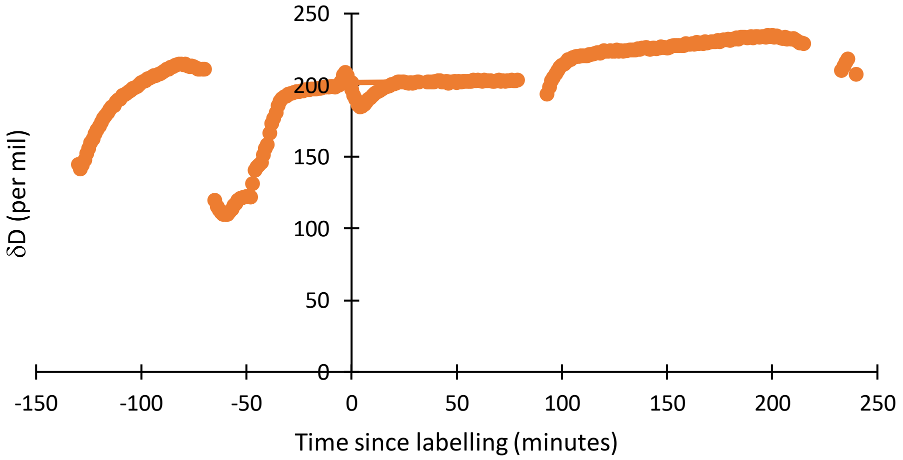


Figure A28. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 5 (control).

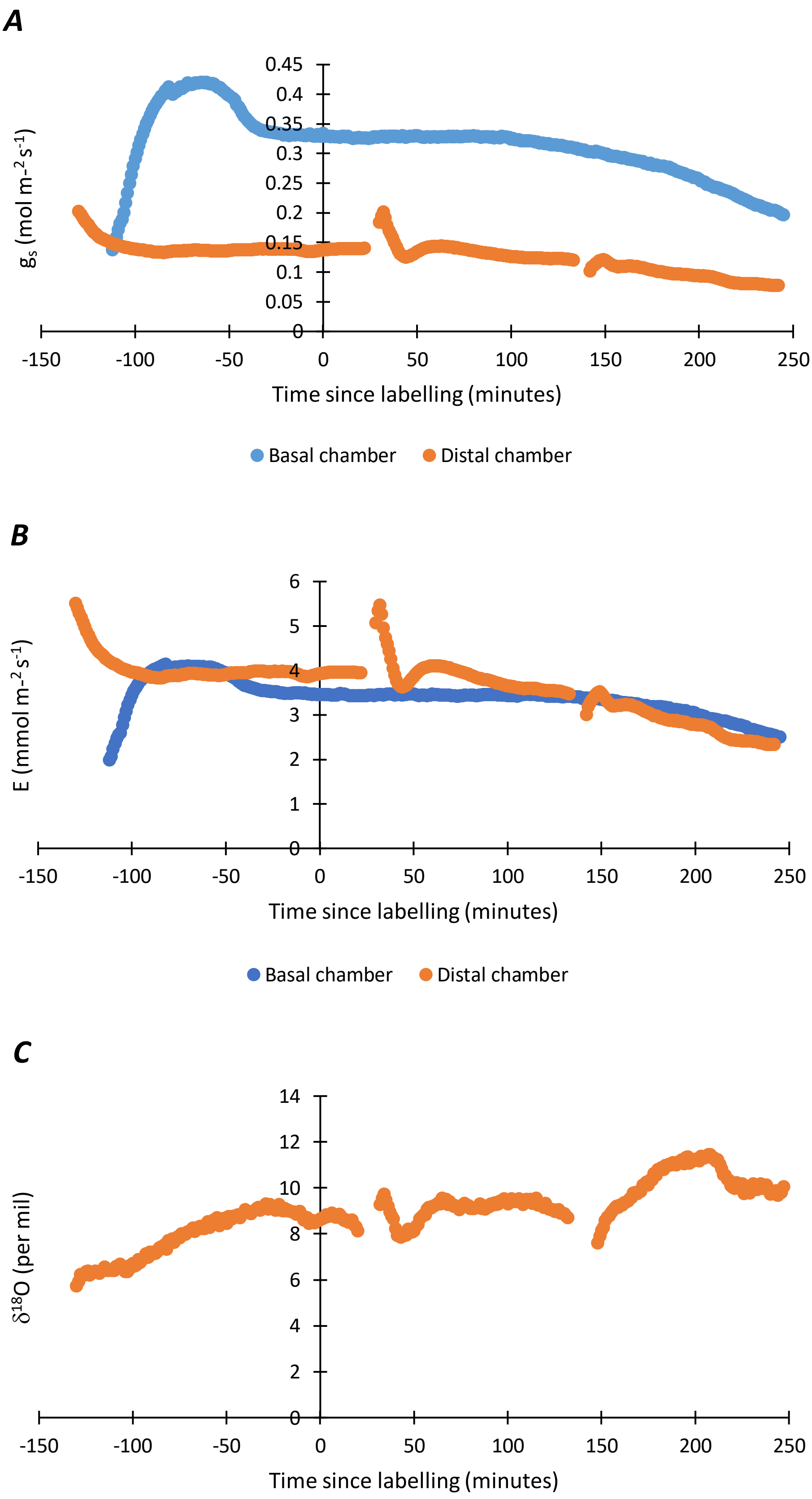


Figure A29. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 6 (control).

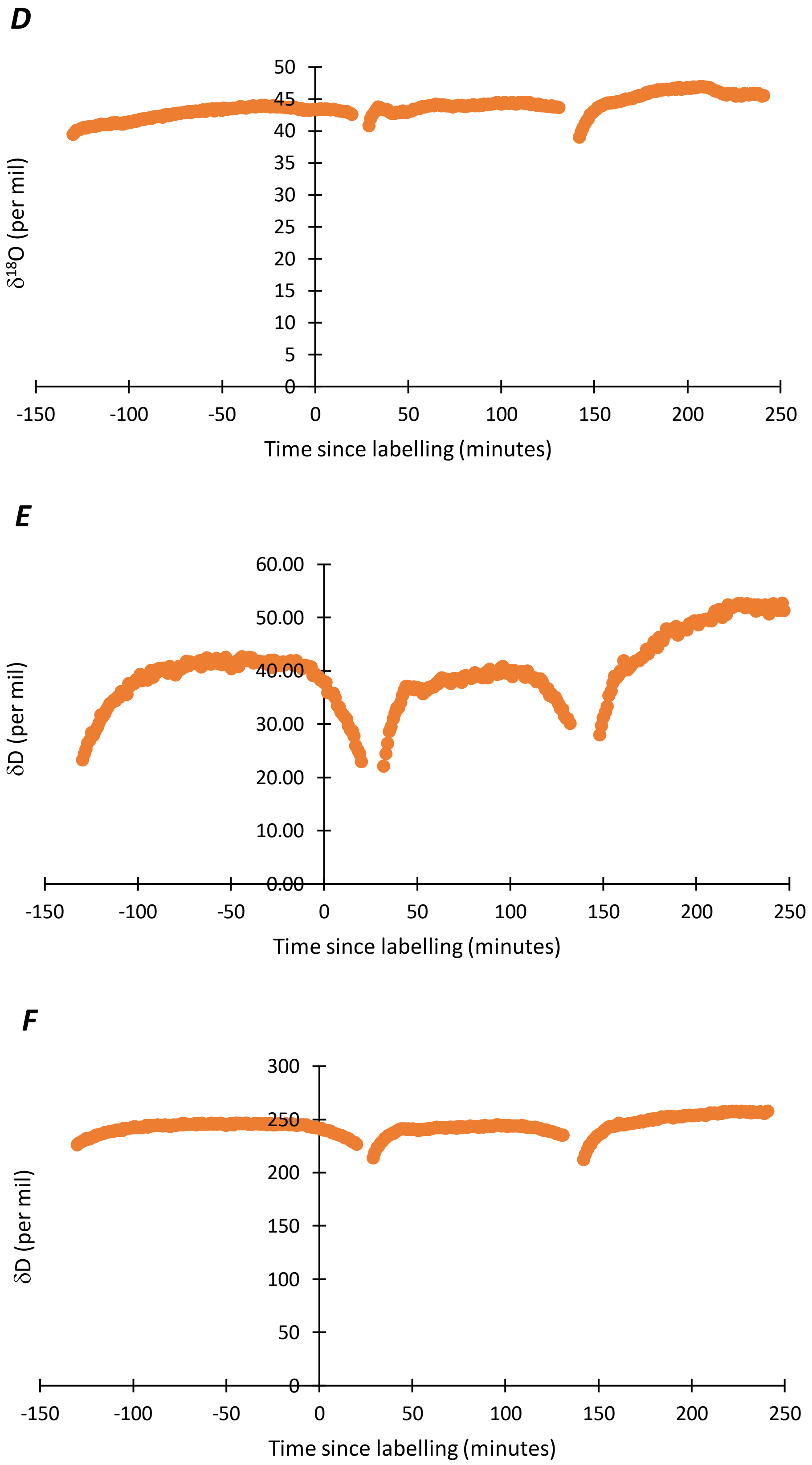


Figure A30. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Oat plant 6 (control).

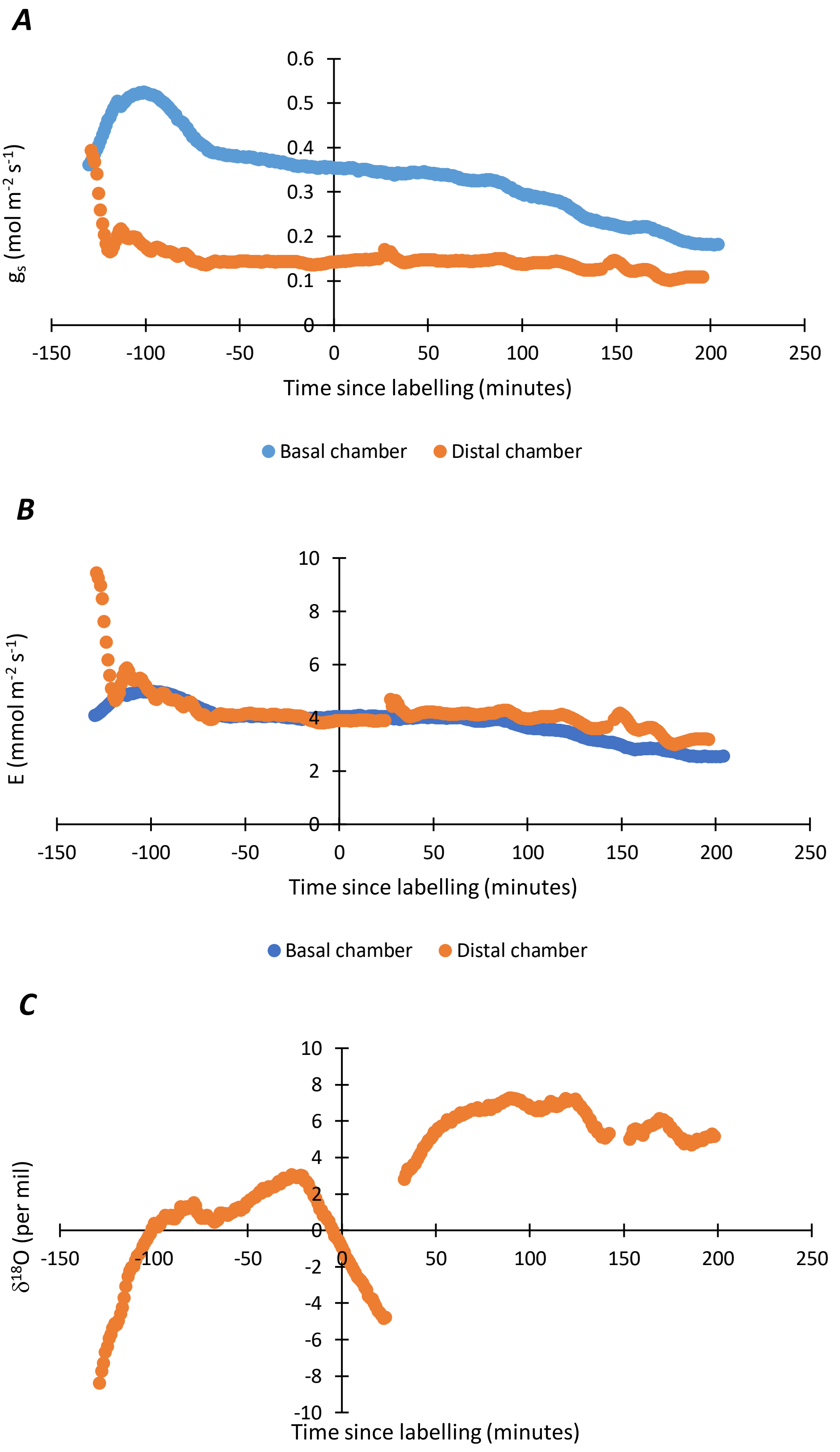


Figure A31. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Oat plant 8 (control).

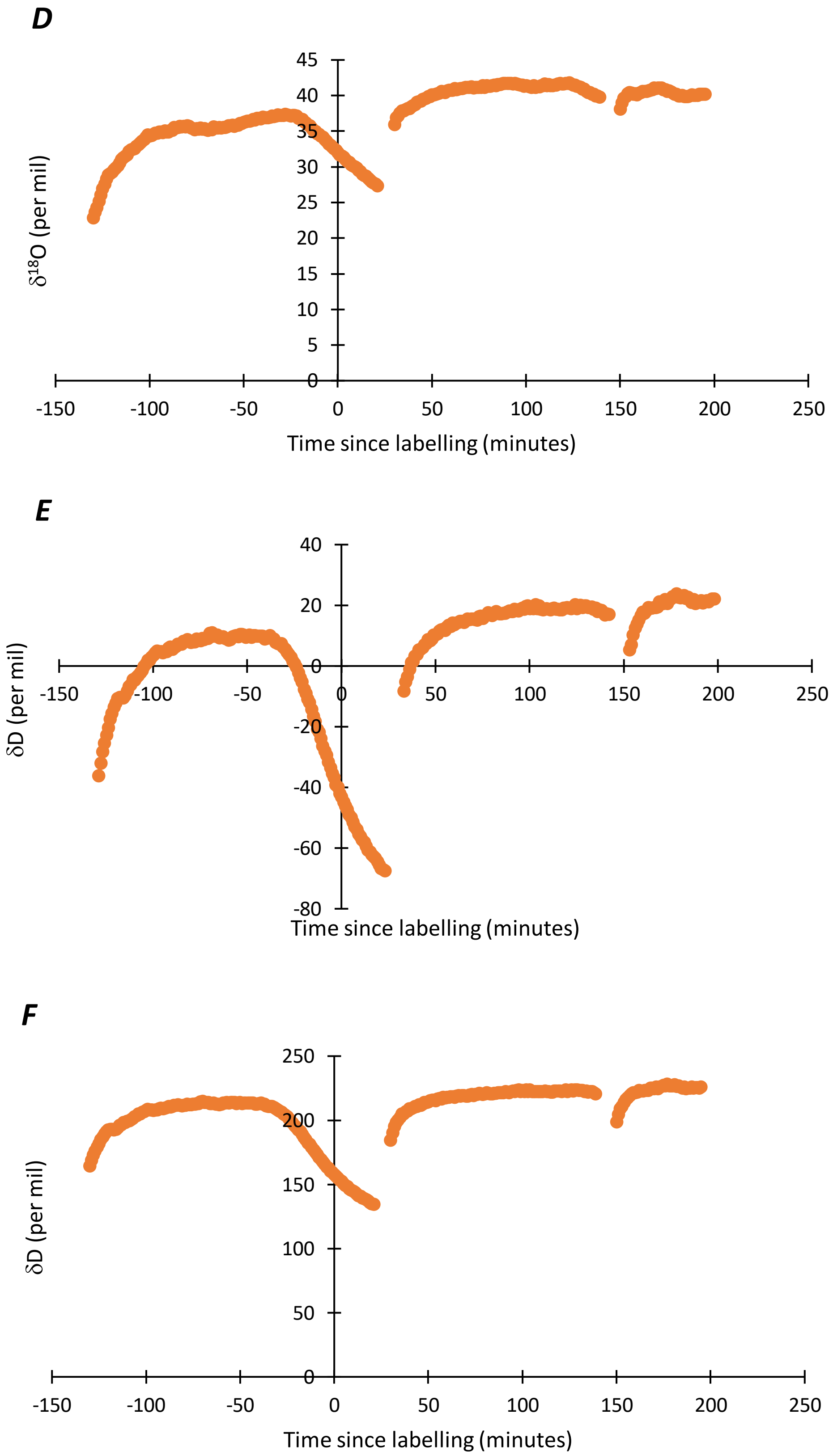


Figure A32. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Corn plant 3 (label).

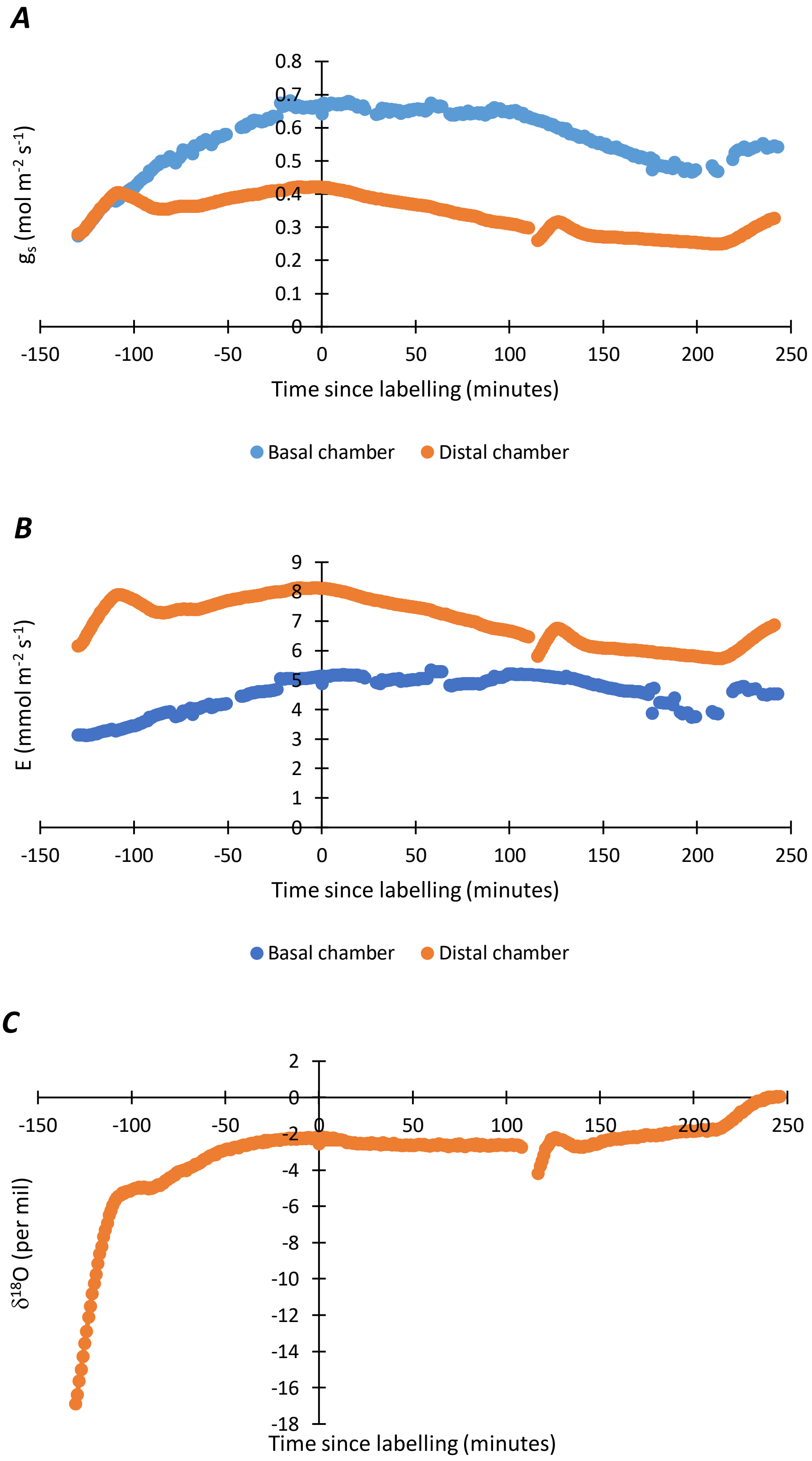
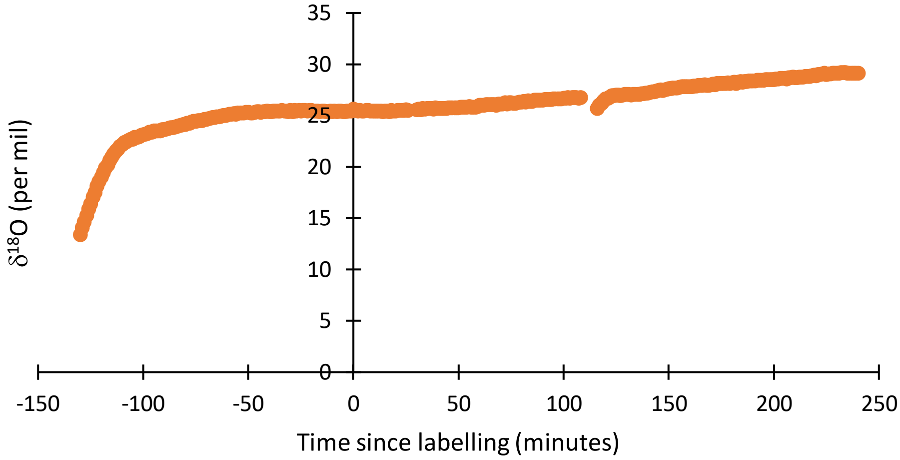


Figure A33. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Sunflower plant 1 (label).

D



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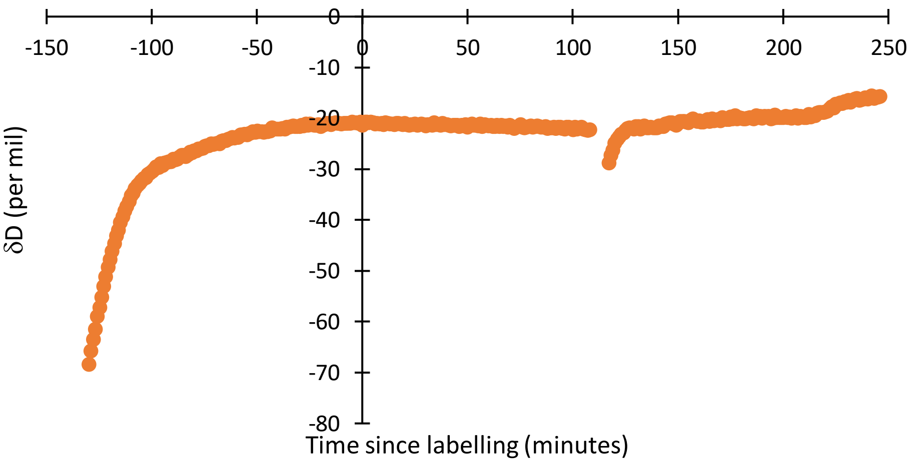

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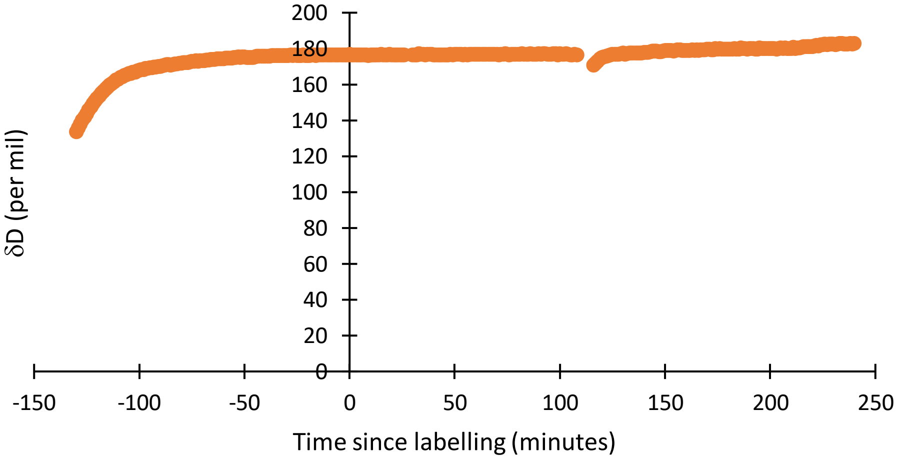

Figure A34. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Sunflower plant 1 (label).

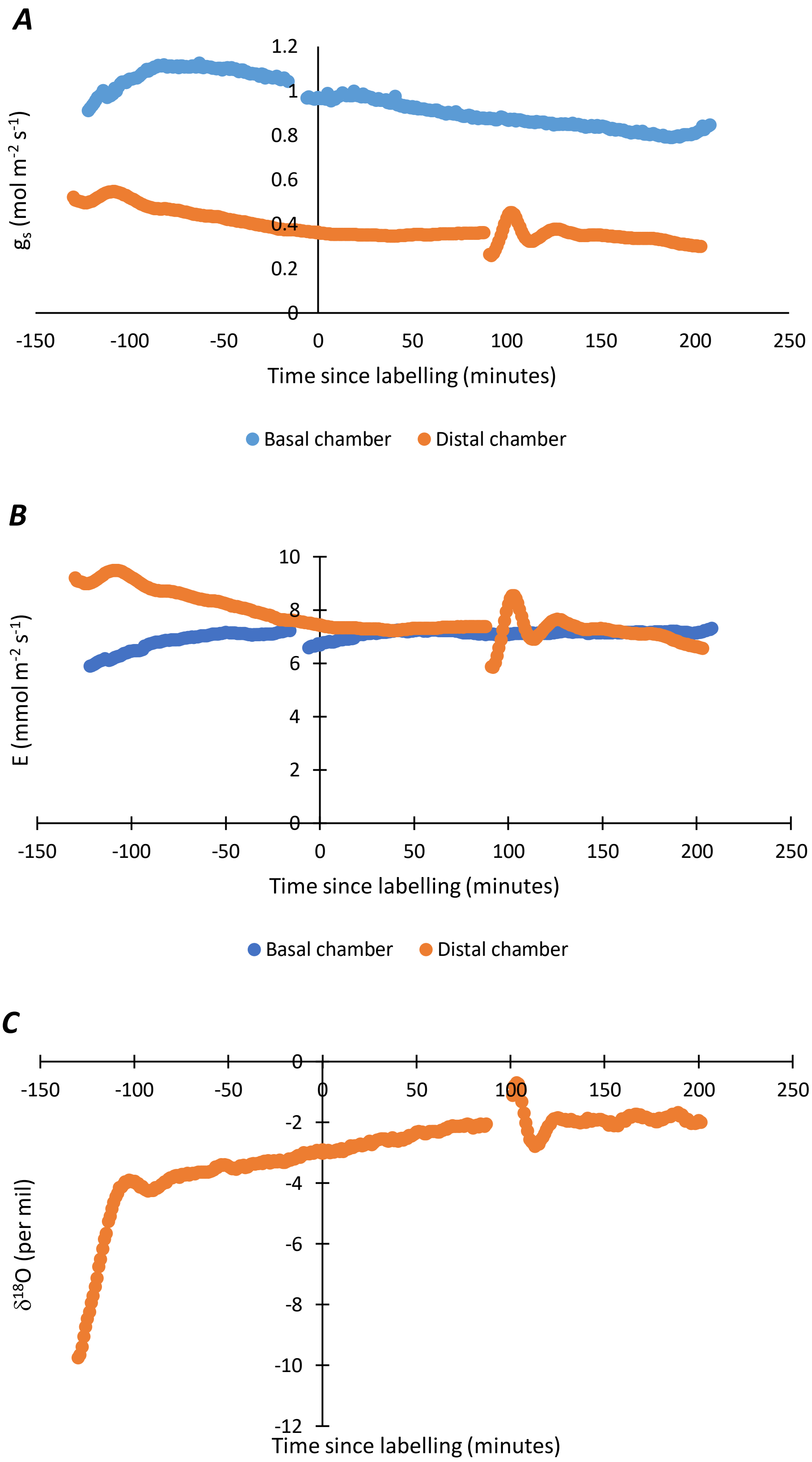

Figure A35. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Sunflower plant 3 (label).

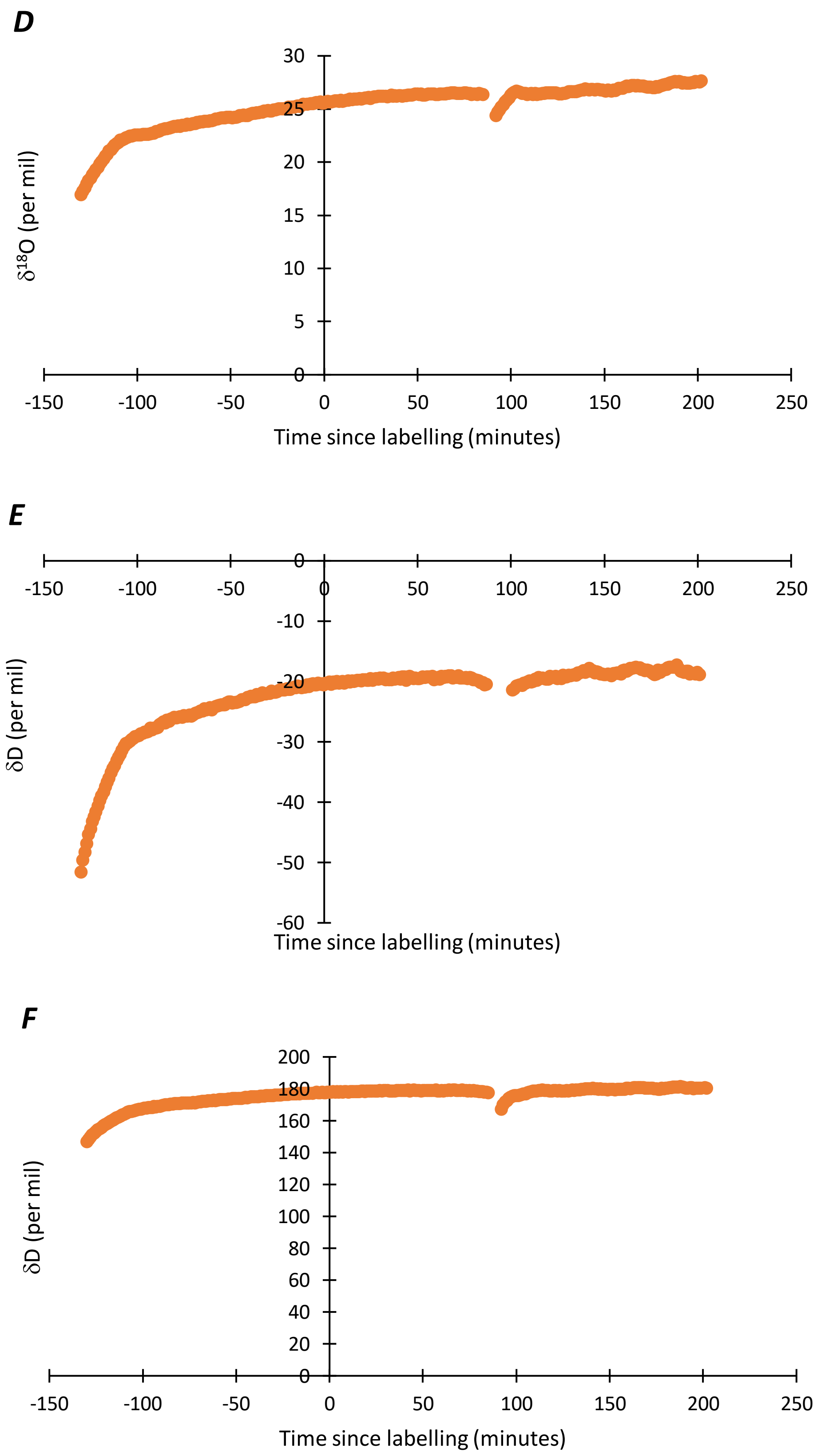

Figure A36. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Sunflower plant 3 (label).

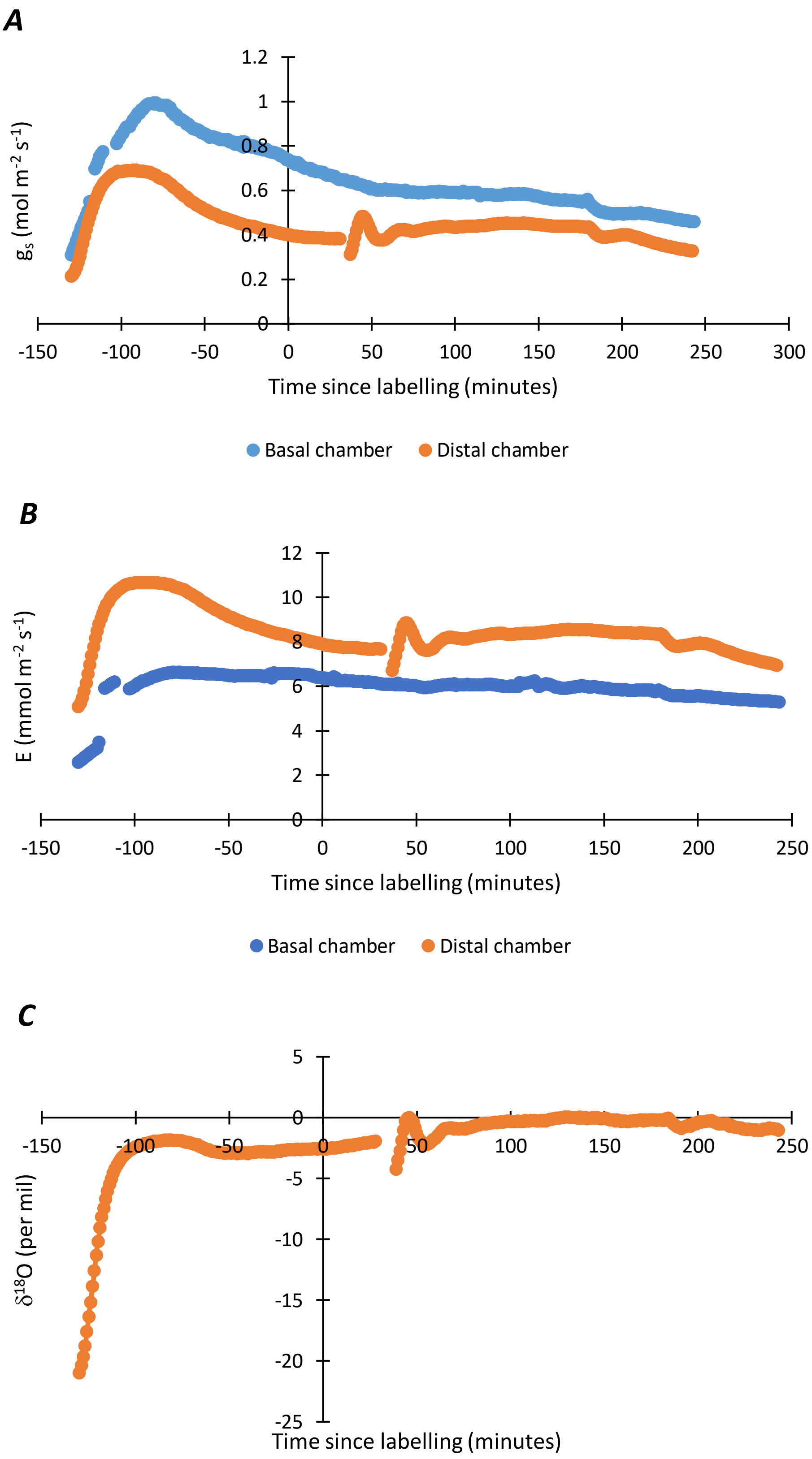

Figure A37. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Sunflower plant 4 (control).

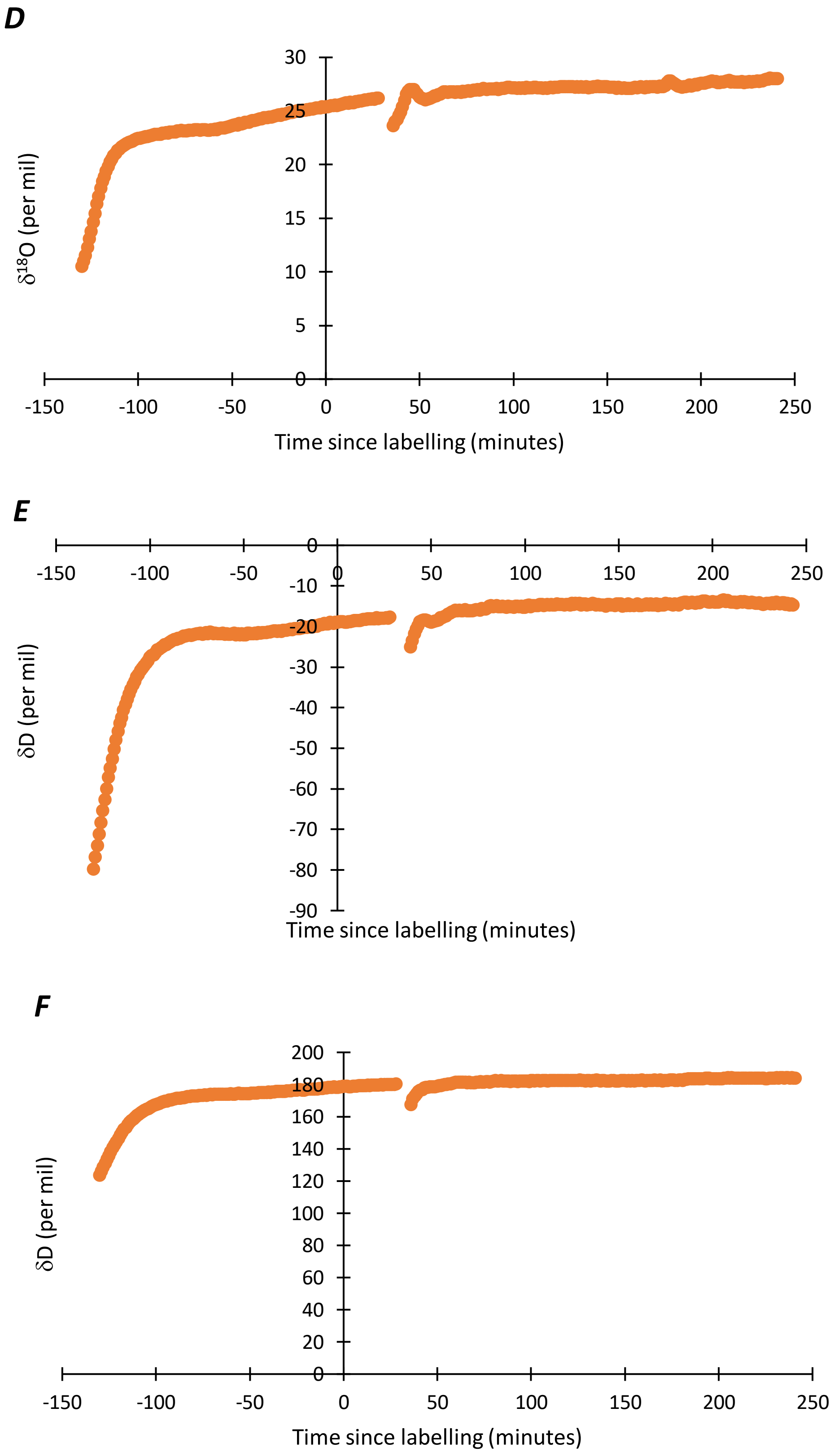

Figure A38. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Sunflower plant 4 (control).

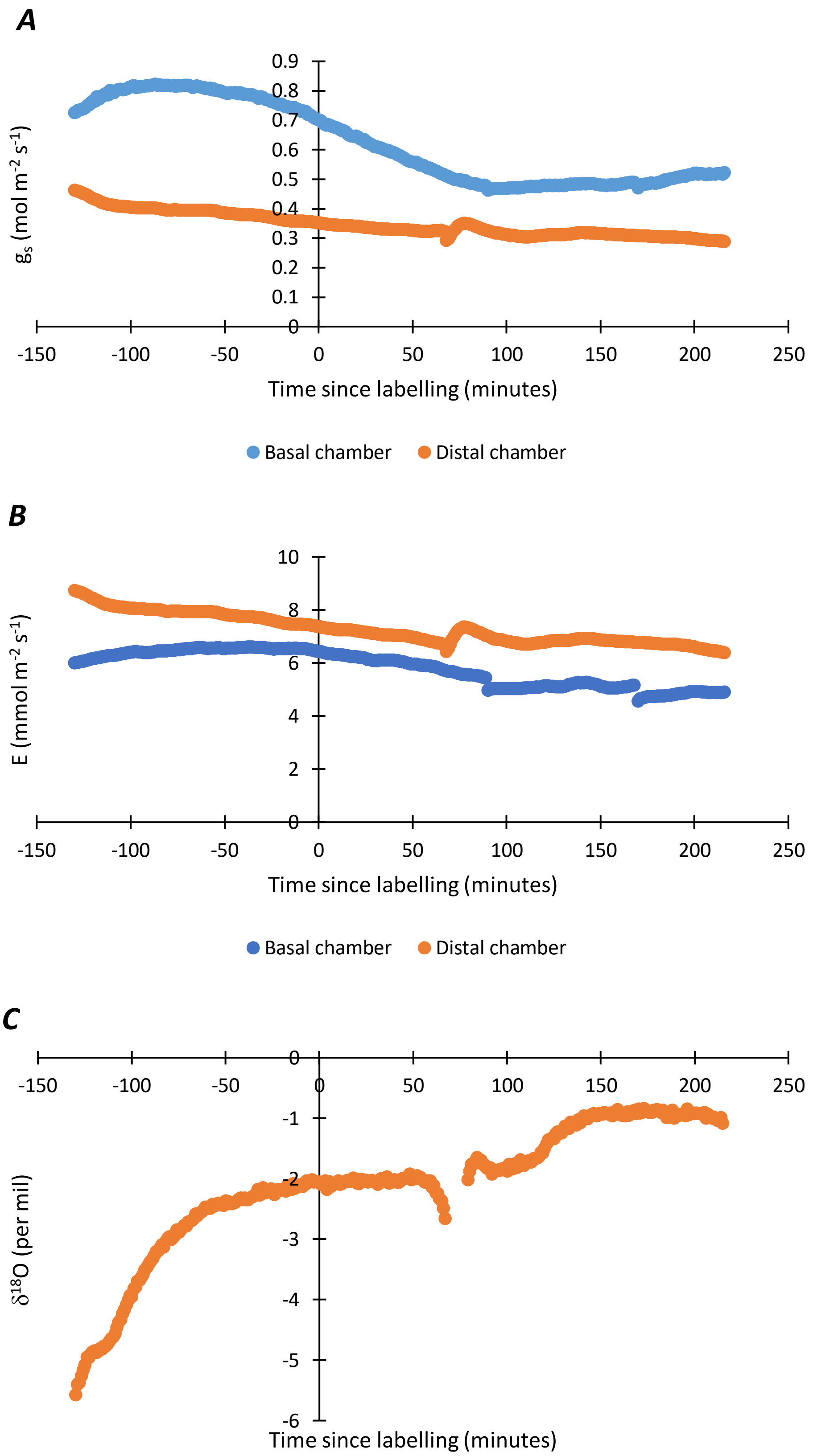

Figure A39. Measurements of stomatal conductance (A), transpiration rate (B) and transpired vapour oxygen isotope composition (C) for Sunflower plant 6 (control).

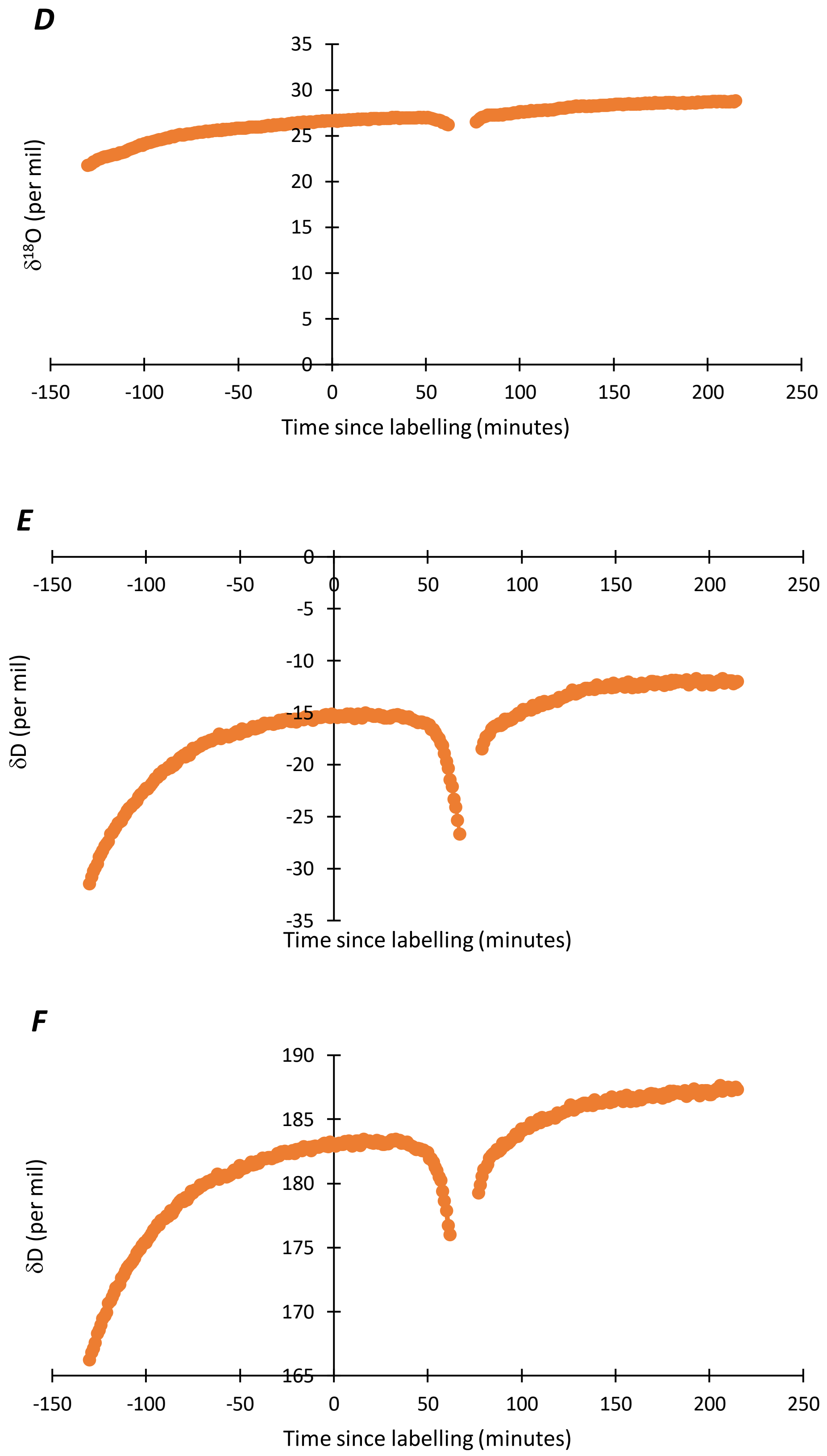

Figure A40. Measurements of leaf water oxygen composition (D), transpired vapour hydrogen isotope composition (E) and leaf water hydrogen composition (F) for Sunflower plant 6 (control).