

The need to decipher plant drought stress along the soil-plant-atmosphere continuum

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Abstract

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The need to decipher plant drought stress along the soil-plant-atmosphere continuum

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Data accessibility

All data and script for data analyses will be made freely available on zenodo upon publication.

Abstract

Lacking comparability among rainfall manipulation studies is still a major limiting factor for generalizations in ecological climate change impact research. A common framework for studying ecological drought effects is urgently needed to foster advances in ecological understanding the effects of drought. In this synthesis, we argue, that the soil-plant-atmosphere continuum, describing the flow of water from the soil through the plant to the atmosphere, can serve as a holistic concept of drought in rainfall manipulation experiments which allows for the reconciliation experimental drought ecology. Using experimental data, we show that investigations of leaf water potential in combination with edaphic and atmospheric drought – as the three main components of the soil-plant-atmosphere-continuum – are key to understand the effect of drought stress on plants. Based on a systematic literature survey, we show that especially plant and atmospheric based drought quantifications are strongly underrepresented and integrative assessments of all three components are almost absent in current experimental literature. Based on our observations we argue, that studying dynamics of plant water status in the framework of the soil-plant-atmosphere-continuum can foster comparability of different studies conducted in different ecosystems and with different plant species and can facilitate extrapolation to other systems, species or future climates.

Introduction

“Under some circumstances, and for some purposes, we can, of course, isolate certain parts of the total system and study only certain modes of water transfer; but a general appreciation of the plant water relations of the whole plant in nature must involve the soil-plant-atmosphere continuum (SPAC)” (Philip 1966)

Rising temperature and changes in the amount and temporal distribution of precipitation are the main symptoms of global climate change (IPCC 2021). Drought events are expected to increase in frequency and severity in large parts of the world including Europe (Böhnisch *et al.* 2021). Changes in precipitation amount and temporal distribution are expected to have strong effects on plants as key primary producers and structuring component in terrestrial ecosystems (Gorton *et al.* 2019). Understanding the biological and ecological responses of plants to drought is therefore paramount for future management of ecosystems and agricultural production (Hoover *et al.* 2018; Matos *et al.* 2020a).

Ecologists have a long-standing interest in drought with research articles including the term “drought” in their title dating back to the 1920s or even beyond (Gorham & Kelly 2018). Sullivan already wrote in (1971) that “the number of publications which are partially or entirely concerned with measurements of plant drought stress is too great to even estimate”. Over the last two decades, drought manipulation experiments have become a major tool in ecological research with hundreds of experiments have been conducted across the globe (Yahdjian & Sala 2006; Gherardi & Sala 2013; Kreyling & Beier 2013). Global experimental networks such as the DroughtNet initiative have been implemented in the last years to study drought effects on ecosystems on a global scale in a coordinated fashion (Knapp *et al.* 2017).

The main objective of all these experiments is to decipher the cause-effect relationships between changing availability of water, plant performance and ecosystem functioning in order to provide a robust basis for

climate change assessments and future impact modelling (Yahdjian & Sala 2006; Kreyling & Beier 2013; Kreyling *et al.* 2018). Observed effects of drought on plants include reductions in net primary productivity and species richness as well as altered carbon cycling (Hoover *et al.* 2018 and references therein). However, results remain often inconclusive and mechanisms of drought responses across ecosystems remain difficult to identify (Wu *et al.* 2011; Wilcox *et al.* 2017; Hoover *et al.* 2018). Based on this prevalent ambiguity, the question of whether system-specific peculiarities or experimental artifacts are contributing to this inconsistency is repeatedly raised in literature (Felton & Smith 2017; Hoover *et al.* 2018).

In this study we re-ask this still relevant and still un-answered question and highlight the advantages of investigating drought effects on plants along the soil-plant-atmosphere-continuum (SPAC) *sensu* Philip (1966). We show that investigations of plant water potential in combination with edaphic and atmospheric drought – the three main components of the soil-plant-atmosphere-continuum – are key to understand the effect of experimentally implemented drought stress on plants. We furthermore show that this integrative approach is almost entirely neglected in current plant-focused drought manipulation experiments. We support this statement with a systematic survey of current literature on drought manipulation experiments and combined this with own empirical data from a rain-out shelter drought experiment we ran during the vegetation period of 2021. The goal of this study is not to question the validity of drought research conducted in the last decades. It is rather to raise awareness amongst researcher about the relevance of a unifying concept for studying drought and to suggest ways in which future studies could be modified to foster synthesis and strengthen mechanistic understanding deducible from ecological drought research.

Methods

The literature search was conducted on Scopus on October 10th 2021 using common terminology for experimental drought research on plants [search term: drought AND experim* AND plant* AND (shelter OR roof OR rain-out)]. This resulted in a total of 234 publications published since the January 1st 2012, thus, covering the last 10 years (see Table S1 Supplemental Material). All publications were screened by eye for the following four criteria, a) was plant water potential measured (Yes/No), b) how was water availability manipulated, c) how was drought characterized and d) what type of response was measured. Additionally, we first classified the type of study objects as community (i.e. multi-species communities with more than 4 species), variety (multiple varieties, lines, ecotypes, populations of one species), multiple species (multiple species but less than or equal 4) and single species (only one study species) and, second, qualified the direction of the investigated response in positive, negative, none (non-significant) and inconsistent (varying directions among the investigated responses within a response category). We differentiated between 9 different response categories, i.e. abiotic, biodiversity, plant biomass, plant chemistry, decomposition, ecophysiology, morphology, phenology and species interaction (see Table S2 Supplementary Material). Publications without artificial drought manipulation (here: 87; e.g. natural occurring drought periods, reviews, studies dealing with questions not related to drought) have been excluded prior to analysis. From the remaining 147 publications, we calculated frequencies of each of the four chosen criteria, grouped on the binary condition if plant water potential was measured or not. For the ease of analysis categories have been homogenized (see Table S3 Supplemental Material).

The empirical data originates from the Hohenheimer Climate Change (HoCC) Experiment, a rain-out shelter experiment which was set up in 2008 at the experimental station Heidfeldhof of the University of Hohenheim (48°42'50"N, 9°11'26"E, 395 m a.s.l., Poll *et al.* 2013). In total 8 plots were assigned into 4 pairs with one sheltered and one ambient control plot. Each plot had an area of 1 m² and was surrounded by a vertical plastic barrier reaching 0.5 m deep into the soil to avoid lateral water flow. During 2021, the experiment focused on the drought response of winter wheat (*Triticum aestivum* L., cv. 'RGT Reform'), which was sown manually at 28th of October 2020. Shelters were installed at the 26th of April 2021 to induce a drought until a soil dryness of 94 % of the volumetric water content of ambient conditions or a distinct increase in leaf water potential below -2 MPa was observed. On the 23rd of June the experimental drought was ended with a single rewetting by applying 25 mm of water per droughted plot. Mean air temperature was 14.1degC and ambient control plots received 138 l in 31 precipitation events during the experimental trial (Station Hohenheim,

48deg42'40"N, 9deg11'46"E, 389 m a.s.l.; retrievable from: <https://www.wetter-bw.de/Agrarmeteorologie-BW>). All plots received a similar amount of fertilizer following common agricultural practice in three applications prior to the experimental drought with in total 130 kg N ha⁻¹yr⁻¹. Plots were weeded manually and pesticide (insecticide and fungicide) was applied once during the experiment to avoid detrimental pathogen infestation.

Plant water status was quantified during the experimental trial once a week by measuring leaf water potential (Ψ_{leaf}) using a Scholander Pressure bomb (PMS Instruments, Model 1500) and by following the standard protocols of Pérez-Harguindeguy (2013). We measured pre-dawn water potential between 5:00 and 5:45 AM prior to sun rise and midday water potential between 12:00 and 12:45 CEST. Pre-dawn water potential is strongly controlled by the soil and indicative for static drought stress in plants whereas midday water potential is predominately controlled by the atmospheric water demand and provides a sensitive indicator on how plants control their water under atmospheric drought. Measurements were intensified (two measurements per week) after plants started to show increasing rates of change in leaf water potential after the 14th of June (7 weeks after the beginning of the drought treatment).

Soil moisture is given as volumetric water content and was quantified by TDR probes (15 cm rods, CS630/CS635, Campbell Scientific Ltd.) every 20 min in 0–15 cm soil depth in all plots. In a subset of plots, additional TDR probes were installed in 15–30 cm depth. Aboveground biomass of the manipulated and control plot was determined at the end of the experimental trial by cutting the inner 3 rows on centred 70 cm ([?] 0,2625 m²). The whole plant was cut near the soil surface. Senescent leaves within the harvested area were added to the harvested biomass. Harvested plant individuals were counted, dried for 7 days at 30 degC to constant weight and weighed.

We investigated temporal dynamics of the coupling of leaf water potential to edaphic drought and atmospheric water demand with simple linear regression models between leaf water potential (Ψ_{leaf}) and Vapor Pressure Deficit (VPD) as well as Ψ_{leaf} and the Volumetric soil Water Content (VWC). We used adjusted R² from these models to quantify the degree of coupling based on a period of 5 consecutive measurement dates. We used this period as the width of a moving window, which we ran over the entire time series of the experimental trial. All analyses were performed in R (v.4.1.0; R Core Team 2021).

The current status and challenges related to rainfall manipulation experiments

During the last two decades, rainfall manipulating field experiments predominantly used passive rain-out shelters to apply drought stress on plants (Kreyling & Beier 2013; Kreyling *et al.* 2017). These rain-out shelters, which are usually placed over intact stands of plants, thereby exclude or reduce precipitation falling into the experimental stands. The manipulated stands are then usually compared to ambient control stands which receive ambient rainfall (Yahdjian & Sala 2006). This method of passive rain exclusion or reduction was originally developed for agricultural studies (Horton 1962) and was then adopted in ecology in the late 1990s (Reynolds *et al.* 1999). Besides full rain-out shelters, also partial rain exclusion by shelters covered with strips of clear plastic gutters were introduced in the early 2000's (Yahdjian & Sala 2002) and are increasingly used in ecological experiments. In all cases, the prime goal of such experiments is to exclude or reduce rainfall for the plant stands under study while all other environmental conditions are supposed to remain unchanged in comparison to the ambient control (Yahdjian & Sala 2006).

The first challenge associated with rain-out shelters and the accompanied assumptions is, that microclimatic conditions beneath the shelters are often modified by the constructions. Such artifacts related to rain-out shelter design in combination with a lack of sufficient controls and biased geographic coverage of precipitation manipulation field experiments have been pointed out by several authors during the last decades (Dugas Jr. & Upchurch 1984; Beier *et al.* 2012; Kreyling & Beier 2013). Unwanted side-effects of rain-out shelters on the microclimate include reduced wind speed, altered radiation or warming (Fay *et al.* 2000; English *et al.* 2005; Vogelet *et al.* 2013; Power *et al.* 2016). Other studies have reported minimal effects of rain-out shelters on microclimatic conditions with no detectible consequences for leaf-level photosynthesis (e.g. Loik *et al.* 2019 for costal Californian plant communities). Thus, the importance of unwanted microclimatic effects of

shelters can be highly site-specific and definitely needs attention.

The second challenge is, that manipulation strength of rain-out shelters strongly depend on the surrounding weather conditions. Plant drought stress is indicated by negative plant water potential as a consequence of high atmospheric suction pressure but low soil water potential. High humidity during rainfall events do not only change the atmospheric water demand and, thus, transpiration of plants in the control but also in the manipulated (sheltered) plots (Kreyling *et al.* 2017). Scenarios such as low soil water availability (edaphic drought) and low evaporative water demand (low atmospheric drought) can occur in nature e.g. in cold deserts but rarely occur in other ecosystems such as temperate grasslands or forests (e.g. Caldwell 1985). Once, low soil water availability and low atmospheric drought do co-occur, they do not induce drought stress in plants. In other systems such as drylands, VPD is always high and soil moisture can be episodic which will have different effects on drought stress emergence in plants. Thus, depending on the weather conditions during the experimental trials (e.g. wet and/or cold weather), rain-out shelters can create artificial conditions in relation to drought which incompletely reflect natural drought conditions usually characterized by low soil water availability but high atmospheric water demand.

Based on incomplete considerations of the evaporative water demand during experimental trials, many studies fail to create drought stress for plants with their precipitation manipulations. This was true for 50% of 564 studies between 1969–2018 reviewed by Slette *et al.*(2019) and 50% of 101 study cases between 1989–2018 that were analyzed by Matos *et al.* (2020b). Although detrimental for interpretation, detailed information on microclimatological artifacts in rainfall manipulation experiments is largely missing or reported incompletely. Especially vapor pressure deficit (VPD) as a reliable measure of atmospheric water demand is neglected in most cases (91% of 65 studies published between 2000 -2014 and analyzed in Kreyling *et al.* 2017). This lack of information with regard to atmospheric water demand becomes especially relevant when assessing effects of drought on carbon sink dynamics. Photosynthetic capacity of plants is closely related to stomatal regulation which in turn is closely coupled to atmospheric water demand (Buckley 2016; McAdam *et al.* 2016). Lower diffusion of CO₂ from the atmosphere through drought regulated stomata is considered to be the major reason behind decreased photosynthetic rate during mild to moderate drought stress (Pinheiro & Chaves 2011) with potentially major implications for global carbon sequestration. A solid characterization of edaphic and atmospheric drought effects on plant water status is therefore imperative to strengthen inferences on future carbon source and sink dynamics drawn from drought experiments.

Our literature survey shows that most of the reviewed studies did not measure plant water potential (85% of 337 study cases, Fig. 1). Full rainout shelters were the most prominent form of drought manipulation in both study groups, those studies which measured plant water potential and those studies which did not (Fig. 1a). The majority of studies quantified drought only using one of the here presented drought categorization categories (84%). Only one study quantified drought using all three categories of the SPAC to quantify the drought, i.e. edaphic, atmospheric and plant characteristics. Drought was predominantly characterized by edaphic factors by using the term “soil moisture” without any further specification (31% and 48% of studies for the two study groups). If specified, volumetric and gravimetric soil water content were the most prominent characterization criteria. At least 14% of the studies measuring plant water potential did use this plant-related parameter to characterize drought. Studies which did not measure plant water potential did mainly focus on biomass (36%) as response variable followed by ecophysiological and morphological parameters to equal shares (both 17% of studies for this study group, Fig. 1c). The share of studies with a focus on ecophysiology was visibly higher for studies that also measured plant water potential (31% of the studies in this group). Most of the studies measured one (39%) or two (48%) categories of response we quantified (see methods). Though two studies quantified four different types of responses, whilst only quantifying drought treatment in one (edaphic) respectively two (temporal and edaphic) different ways. Single species studies are most frequent (35%), whilst in total 47 out of the 147 investigated studies (32%) investigate communities, followed by varieties (19%) and multiple species (14%) studies. Approximately 50% of the studies (which did not measure plant water potential) investigating biomass or biodiversity report inconsistent or non-significant impact of drought, while the expected negative effect is reported in 36% (biodiversity) respectively 46% (biomass). If water potential was measured, the share of negative results

on biomass increases to 63%, whilst none or inconsistent reported effects of drought application decrease markedly (37%). All this implies, that plant water potential as a measure of plant water status is generally neglected in experimental plant ecology for drought characterization as well as response variable but can provide a solid approach to quantify the effectiveness of drought applications. This lack of attention with regard to the ecohydraulic effects of rainfall manipulation is reinforced by the fact, that ecophysiological and ecohydraulic effects measured beneath rain-out shelters are often not investigated under opposing ambient weather conditions (Kreyling *et al.* 2017). We argue that all this leads to an incomplete picture in current literature about how drought affects plants – which is another main or even the most important reason for the inconsistency existing among rainfall manipulation studies with regard to drought effects on plants.

Drought and the soil-plant atmosphere-continuum

Proper characterizations of drought and its effects on plants are paramount considering the increasing frequency and severity of drought events accompanying global climate warming. Climatologists have been discussing about definitions for drought for decades and identified a variety of different types of drought relevant for different aspects of our socio-economic and ecological systems (e.g. meteorological, agricultural, hydrological or socioeconomic drought; Slette *et al.* 2019). Numerous indices have been developed over the years to improve objectivity and consistency in the characterization of drought conditions (e.g. Zargar *et al.* 2011; Svoboda *et al.* 2016). However, any universal definition of drought is accepted among climatologists to be elusive and impractical at the same time (Redmond 2002; Lloyd-Hughes 2014).

In ecological literature, definitions of drought such as “an episodic deficit in water availability that drives ecosystems beyond thresholds of vulnerability, impacts ecosystem services, and triggers feedbacks in natural and/or human systems” have been proposed (Crausbay *et al.* 2017). However, little consistency exists among ecologists about how to define drought, conceptually or operationally and minor shares of studies explicitly define drought for their study (approx. 32% of 564 studies between 1969 and 2018 reviewed by Slette *et al.* 2019). Similarly, various definitions exist for plant drought stress based on soil moisture, evapotranspiration, canopy temperature, stomatal conductance, and plant water potential (Zhang *et al.* 2021). All this diminishes comparability of these studies and, thus, large-scale synthesis of the findings (Fraser *et al.* 2013). A common framework for studying ecological drought effects seems to be urgently needed to foster advances in ecological understanding. Standardized drought indices such as the standardized precipitation evapotranspiration (SPEI) index have been proposed as a measure of drought intensity independent from differences in annual precipitation between different sites (Matos *et al.* 2020b). However, the SPEI index does only characterize the abiotic boundary conditions of drought but misses out plant- and ecosystem specific differences in how these boundary conditions translate into plant drought stress, which is actually the essence of almost all drought manipulation studies in plant-focused ecology.

Water is an essential resource for and component of plants not least because plants inevitably lose water when they assimilate carbon from the atmosphere to maintain growth and secure their fitness. We argue, that the soil-plant-atmosphere continuum (SPAC) provides a well-established concept on water relations in ecosystems that can provide a standardized framework for ecological research on drought effects. The SPAC concept basically describes the flow of water from the soil through the plant to the atmosphere, thus, the transpiration stream (Passioura 1982). The evaporative water demand of the atmosphere coupled with the plants need to acquire carbon provide the driving forces of this transpiration stream (Philip 1966). The soil-plant-atmosphere continuum thereby covers edaphic and atmospheric drought as well as the plant’s response to both in terms of its water status and drought stress. We argue, that a general and objective characterization of drought stress in plants can only be fostered by collecting data on all three components of the soil-plant-atmosphere continuum, namely soil moisture, atmospheric water demand and plant water potential in different organs of plants (such as leaves or the stem). We will reinforce our argumentation by using empirical data from one of our own rain-out shelter drought experiments.

Lessons from a rain-out shelter experiment

We conducted a drought experiment in Southern Germany (Baden-Württemberg) on winter wheat – one

of the most important crops in Central Europe – where we completely excluded rainfall for 8 weeks (from April to June 2021) using rain-out shelters. Despite these 8 weeks of complete rainfall exclusion, we did not observe any effect on above ground biomass, i.e. no significant difference between the total above ground dry biomass of the rainfall exclusion and control plots ($p = 0.575$, $F = 0.329$, Fig. 1). Volumetric soil water content of the manipulated plots decreased below 10 % after 2 weeks at 0-15 cm depth with slow but steady decreases afterwards and reached a minimum of 6 % after 8 weeks (Fig. 2). VWC remained higher in deeper soil layers with 18 % at 15-30 cm depth after 8 weeks of rainfall exclusion. This set of observations could lead to the conclusion, that extensive drought does not affect biomass production, thus, yield in winter wheat.

A different picture emerges when focusing on the plant water status over the course of the drought experiment. Pre-dawn leaf water potential indicative for static drought stress showed no difference between drought treatment and control until day 48 (week 7, 06/14/2022) of rainfall exclusion but diverged afterwards with manipulated plants becoming significantly more drought stressed; Fig. 2a). This divergence of leaf water potential went along with a warm and dry weather period after a rather cold and wet period as reflected by comparably low vapor pressure deficit during the first 5 weeks followed by increases in VPD which was especially pronounced in the last week of the artificial rainfall exclusion period (see Fig. 2, Supplemental Material Fig. S1). The other two less pronounced warm periods right at the beginning of the experimental trial and in week 6 (01-07 June 2022) did not show any effects on plant drought stress. For midday water potential we did not observe such divergence between manipulation and control. Both groups of plants were quite closely following the temporal variation in VPD showcasing the strong effect of atmospheric water demand on plant water status.

We observed pre-dawn leaf water potential of the manipulated plants to be increasingly closely coupled to VPD over the course of rainfall exclusion whereas coupling decreased after the experimental plots were irrigated again (red lines in Fig. 4a). This coupling between atmospheric water demand and plant drought stress was less pronounced for the ambient control plants (blue lines in Fig. 4a). For midday leaf water potential coupling to atmospheric water demand successively increased over the course of rainfall exclusion and decreased after rewetting took place with no difference between control and manipulation (Fig. 4b). Coupling of pre-dawn leaf water potential and volumetric soil water content increased for the manipulated plants over the course of rainfall exclusion whereas coupling decreased after the experimental plots were irrigated but was overall very weak for the ambient control plants (Fig. 4c). Midday leaf water potential of the manipulated plants was more loosely coupled to volumetric soil water content but increased towards the end of the experiment (Fig. 4d). All this indicates, that drought stress in plants imposed by edaphic drought is closely coupled to atmospheric water demand and that this coupling strongly increases with increasing atmospheric drought (VPD). Drought stress in plants emerges under a combination of edaphic and atmospheric drought (i.e. low soil moisture and high atmospheric water demand) with the role of the atmosphere in triggering plant drought stress seeming to increase with increasing edaphic drought. Thus, VPD seems to be the driving force of plant drought stress emergence during the day in mesic ecosystems. However, the relative importance of soil vs. atmospheric drought driving the emergence of drought stress in plants will definitely vary across ecosystems. Soil drought rather than atmospheric water demand will probably be of prevalent importance e.g. in dryland systems. The importance of edaphic vs. atmospheric drought will furthermore strongly depend on the time scales considered. Edaphic drought can take weeks to months to show effects on plant water relations when e.g. atmospheric water demand is low whereas high VPD will immediately induce stomata closure and, thus, reduce gross primary production. Deciphering the relative contributions of soil and atmospheric drought in driving plant water relations in a standardized framework such as SPAC could therefore be a major step forward to increase compatibility of drought experiments across different ecosystems.

The urgency to quantify plant drought stress along the soil-plant-atmosphere continuum

The results from our study in combination with our literature survey and the outcomes of other methodological studies on rainfall manipulation experiments clearly show, that lacking information on atmospheric conditions such as weather conditions during the experiment in general and on atmospheric water demand

in particular can hamper comparability of and knowledge gained from drought manipulation studies using rainout-shelters. This seems to be especially true when weather conditions are cool and wet during the trials which lowers atmospheric water demand (cf. Kreyling *et al.* 2017). Consequently, edaphic drought is less likely to impose drought stress to plants under such conditions. On the other hand, rainy periods have been proposed to be the only possibility to create effective differences between rain-out shelter manipulations and ambient control conditions especially in drought prone regions because natural drought during the experimental trial can cause drought responses in the control (Kreyling *et al.* 2017). This seems to put field-scale, rainfall manipulation experiments in a dilemma and calls for context-independent approaches to plan and conduct such studies. Plant water status, quantified e.g. by plant water potential can provide such a context-independent, plant focused index of drought stress. We argue, that the joint interpretation of plant water status in combination with the other two components of the soil-plant-atmosphere continuum, soil water availability and atmospheric water demand, will provide a more holistic picture of drought and drought stress in plant focused studies. Such exhaustive investigations are paramount to minimize misinterpretations and increase at the same time comparability among studies. We will discuss every component of the soil-plant-atmosphere-continuum in more theoretical and methodological details in the following sections.

Soil water availability

Plants predominantly get water from the soil. Soil moisture, measured e.g. as volumetric or gravimetric water content are widely used metrics to characterize the edaphic water supply of plants (Zhang *et al.* 2021, our own literature survey). Volumetric soil water content (VWC) is often measure by Time Domain Reflectometry (TDR) sensors whereas gravimetric soil water content is usually determined by weighing and drying defined soil samples in the lab. However, both measures quantify soil moisture which cannot be directly translated into water accessibility for plants. Soil matric potential is stated to give a more realistic characterization of soil water availability to plants as it quantifies the force a plant would have to apply to extract water from a given soil with its specific soil properties (particle size distribution and pore space, Yadvinder-Singh *et al.* 2014). Furthermore, soil matric potential is measured in the same unit as plant water potential and VPD, which facilitates integrated assessments along the soil-plant-atmosphere continuum. Soil matric potential can be measured by tensiometers or other matric potential sensors. Tensiometers are limited to moderate drought but stop functioning at pF 3 (-10^3 hPa). For measurements below this threshold, other soil matric potential sensors based on e.g. impedance-based determination of the capacitance can be used (see e.g. Jackisch *et al.* 2020 for the comparison of different sensors). Furthermore, water retention curves can be used to translate VWC into soil matric potential for a given soil. Water retention curves are commonly referred to as the relationship between matric potential and volumetric water content for soil with given structural and hydraulic characteristics and defines a critical soil hydraulic property especially when it comes to dynamics in soil drying and rewetting (Dane & Lenhard 2005; He *et al.* 2021). Water retention curves can be determined in the lab for a given soil following standardized procedures (e.g. Kanzari *et al.* 2012; Hardie *et al.* 2013). Furthermore, water retention curves can be defined by *in situ* simultaneous sensor measurements of volumetric soil water content and soil matric potential. However, these field measurements are reported to be less reliable than the time consuming laboratory procedures due to measurement errors originating from poor hydraulic coupling of the sensors including unmatched spatiotemporal resolutions and/or incompatible measurement ranges (He *et al.* 2021).

Atmospheric water demand

Atmospheric water demand measured by e.g. vapor pressure deficit (VPD) plays an equal or even more important role than soil water availability in regulating plant water use (Zhang *et al.* 2021). Stomatal regulation of plants is closely linked to VPD with plants closing their stomata to prevent excessive water loss via transpiration when VPD is high. High atmospheric water demand during periods of drought stress can therefore represent a significant constraint on plant carbon uptake and water use in ecosystems independent of edaphic conditions (Novick *et al.* 2016). This high importance of vapor pressure deficit relates to the sensitive response of stomatal guard cells, which regulate stomatal opening and function as a kind of humidity sensors which are able to detect and respond to the difference in water potential inside and outside the leaf (Lange

et al. 1971). This mechanism can cause stomata to open under low atmospheric water demand (i.e. high air humidity and/or low temperatures, ergo low VPD) even when soil water availability is low due to e.g. experimental rainfall exclusion (Kreyling *et al.* 2017).

Despite its high physiological relevance for plant functioning, vapor pressure deficit is often overlooked in hydrologic and climate science and is chronically neglected in ecological climate change impact research on plants (see Fig. 1). Many rainfall manipulation experiments draw conclusions about ecological response to drought stress, even though VPD is unaffected or not even considered in the experiments (Beier *et al.* 2012; results from our own literature survey). VPD can be calculated from standard metrological quantities such as air temperature and relative humidity or air temperature and dew point (Jones 2013). Climatic variables are ideally measured on-site, i.e. in the vegetation stands using climate data logger insulated against direct radiation (Foken 2021). However, standardized meteorological measurements conducted at meteorological stations close by the experimental site seem to be sufficiently representative as the analyses of our own empirical data, especially the coupling of Ψ_{leaf} and VPD, demonstrates.

Plant water status

Atmospheric water demand pulls water from the soil through the plant to the leaves. This causes water in the transpiration stream to be under negative pressure and, thus, plant water potential to become negative (Pockman *et al.* 1995; Vesala *et al.* 2017). Plant water potential is commonly assumed to be a meaningful measure for plant water status at any time during the diurnal course or the phenological development of plants. Frequent measurements of plant water potential (at least twice a week) can provide an accurate representation of the drought stress history of plants over the growing season (Karamanos & Papatheohari 1999).

Diurnal patterns of leaf water potential are closely coupled to the diurnal pattern in atmospheric water demand and the stomatal response linked to it. The diurnal pattern usually describes a sinusoidal dynamic with water potential reaching its maximum (least negative values) in the early morning just before sunrise and its minimum (most negative values) about midday before it starts to increase again in the afternoon (Sellin 1999). Pre-dawn water potential is used in many ecophysiological studies as measure of bulk soil water availability or even as a proxy for soil water potential at the root surface and can therefore serve as a measure of static (edaphic) drought stress in plants. This is based on the assumption that soil and plant water potential come into equilibrium overnight due to low atmospheric water demand and closed stomata (Sellin 1999 and references therein), conditions that characterize many ecosystems and plant species (except e.g. CAM plants).

Whereas pre-dawn water potential is strongly controlled by the soil, midday water potential is predominately controlled by the atmospheric water demand and provides a sensitive indicator on how plants control their water status under environmental conditions capable of causing detrimental drought damage such as xylem cavitation. Water potential of leaves or twigs is often measured via portable pressure chambers which provides a robust approach for field and lab measurement. Leaf or stem psychrometers provide an alternative approach for automated, continuous measurements in the field or lab. Besides plant water potential, stomatal conductance is considered to be among the most meaningful measures to quantify instantaneous drought stress of plants caused by soil water deficit and atmospheric dryness (Sullivan 1971; Zhang *et al.* 2021).

Some authors argue against using plant response to drought such as plant water potential as primary evidence for drought (e.g. Slette *et al.* 2019). However, drought is a vague term in ecological research with multitudes of meanings and a general lack of definitions which hampers comparability of the outcomes of rainfall manipulation experiments (Fraser *et al.* 2013; Slette *et al.* 2019; Zhang *et al.* 2021). In contrast to the multitude of different explicit or implicit definitions of drought, plants' response to manipulated rainfall can set a common ground for experimental work – at least for plant-focused research. We therefore argue, that a focus on response dynamics in plant water status in relation to changes in the key environmental drivers, i.e. soil water availability and atmospheric demand provides a more mechanistic and context-independent way to study plant water relations in an increasingly changing world.

Conclusions

Advances in ecological understanding do rarely occur by individual studies but mainly by research consensus and synthesis (Knapp *et al.* 2004). Comparability of measurements across experiments as well as the representativeness of experimental results for global ecosystems, current climatic conditions and the forecasted precipitation changes are of prime importance for ecological climate change research (Beier *et al.* 2012). The lack of comparability in drought definitions and drought quantifying parameters among studies are the major limiting factors for generalizations across ecosystems and study species (Vicca *et al.* 2012; Kreyling *et al.* 2017). In this study, we argue for a more holistic approach to study drought emergence and effects as a strategy to reconcile experimental ecology focusing on drought. We argue, that studying dynamics of plant water status in the framework of the soil-plant atmosphere-continuum can foster comparability of different studies conducted in different ecosystems and with different plant species and can facilitate extrapolation to other systems, species or future climates. Such measurements repeated over the course of an experiment will allow to develop reaction norms for plant drought stress responses in relation to changing edaphic water availability and atmospheric water demand – the two main abiotic drivers of water fluxes in ecosystems. Such reaction norms will reflect the relevant processes of plant drought responses and can provide a solid, mechanistic basis for earth system models as prediction tools necessary to develop mitigation and adaptation strategies for the upcoming, global climatic changes (Kreyling *et al.* 2018).

Mechanistic understanding about the relative contribution of edaphic and atmospheric drought to drought stress emergence in plants will provide a sound bases for process-based modelling of plant water relations under different environmental conditions (e.g. different ecosystems). Recently developed modelling frameworks on soil-plant water relations, such as the one of Carminati and Javaux (Carminati & Javaux 2020), should therefore be extended by the atmospheric component and scaled up from individual plant to plant community or ecosystem level. Mechanistic understanding and process-based modelling of soil-plant-atmosphere dynamics will furthermore facilitate our understanding on ecosystem carbon dynamics because of the close links between plant water relations and photosynthetic carbon acquisition. Coupling soil-plant-atmosphere hydraulic models with mechanistic models on carbon and nutrient acquisition and allocation and species distribution modelling (e.g. Higgins *et al.* 2012) will allow scaling from the individual plant level to plant community and ecosystem level. Such coupled modelling approaches will furthermore allow to account for different timescales at which edaphic and atmospheric drought affects plant water relations and carbon acquisition. Experimental approaches, where atmospheric water demand is manipulated on community level (see e.g. Aguirre *et al.* 2021) as well as novel approaches to remotely sense drought stress in plant communities or on ecosystem level (Avetisyan *et al.* 2021; De Cannière *et al.* 2022) will be imperative for upscaling and to validate model predictions.

As stated above, we do not argue, that all components of the soil-plant-atmosphere continuum are equally important in all ecosystems and, thus, have to be considered to a full extent in all drought studies. Individual components, such as soil moisture or atmospheric water demand might be more or less relevant drivers of plant drought stress in e.g. mesic ecosystems versus drylands. However, we argue that SPAC puts all relevant components of water relations in ecosystems in a theoretical and quantifiable framework and, thus, should be considered as helpful tool to plan and conduct ecological drought experiments. Empirical investigations of soil and atmospheric drought in combination with quantitative approaches to assess plant drought stress are evidently more labor intensive than the classical, one-in-time measurements often conducted in drought experiments. We do not argue, that every future experiment has to conduct such measurements. However, we argue, that the gain of fundamentally different insights into ecological dynamics and the chance to prevent false conclusions will in many cases outweigh these additional costs. Two thirds of the studies we reviewed were conducted on a rather small amounts of species ([?] 4). Researcher conducting such studies would have the opportunity to track plant water potential to quantify drought stress within their study without unmanageable amounts of additional labor. For community studies with many species, it might not be feasible to track every single species, though tracking the most abundant or the species of special interest might offer a way to integrate the quantification of plant drought stress within community-based approaches. Generally, we argue that measures based on plant water status (whether leaf water potential or remotely

sensed indices) provide a robust way to document the achieved treatment effect and thereby provide the basis for a justifiable interpretation of results. We generally conclude, that ecological drought research needs a unifying, theoretical framework such as the soil-plant-atmosphere continuum to be rigorously implemented in experimental planning and modelling when aiming for generalization.

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Figure legends

Figure 1. Methods of drought application (a) and characterization (b) and measured responses (c) in rainfall manipulation experiments using full or partial rain-out shelters. Based on 147 published studies between 2012 and 2022. Percentage values at the outer rings depict the shares of the different drought application and characterization methods as well as response variables for each of the two study groups, i.e. studies that

measured plant water potential (dark grey in the inner circles) and studies that did not (light grey circles). All proportions >10% are labelled.

Figure 2. Aboveground biomass of winter wheat plants (dry weight in kg/m²) grown under rainfall exclusion (drought) imposed by rain-out shelters (red) and under ambient control conditions (blue).

Figure 3. Temporal development of pre-dawn (a) and midday (b) leaf water potential of winter wheat plants grown under rainfall exclusion (drought) imposed by rain-out shelters (red) and under ambient control conditions (blue) in relation to dynamics in atmospheric water demand (vapor pressure deficit depicted as black, solid lines). Shown are mean (dots) and standard deviation (error bars) of 4 measurements of leaf water potential per date. Significant differences between drought and control are indicated by asterisks. Volumetric water content of the upper soil (0-15 cm depth) are depicted over the course of the experiment by the colored, solid lines (drought treatment: red and control: blue, periods with dashed lines indicate missing values). Grey shaded area indicates period of experimental implied drought by rain-out shelters.

Figure 4. Coupling of atmospheric water demand (Vapor pressure deficit, VPD, a and b) and edaphic drought (volumetric soil water content, VWC, c and d) with leaf water potential of winter wheat plants grown under rainfall exclusion imposed by rain-out shelters (red) and under ambient control conditions (blue). Intensity of coupling is quantified as adjusted R² of regression models between VPD or VWC and leaf water potential calculated for a moving window with a window width of 5 successive measurements in time. Dates are starting dates of the respective time windows. Curves were fitted using a third-order b-spline with internal cross-validation.





