

Chlorophyll fluorescence, predawn water potential and photosynthesis in precipitation pulse-driven ecosystems – implications for ecological studies

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Summary

1. A major research focus in population and community ecology is to establish a mechanistic understanding of plant interactions and demographic responses. The first step towards this mechanistic approach relies on understanding the differences in stress caused by different environmental conditions. Leaf-level photosynthetic rate (A) within and among plant populations provides important insight into population and community processes, but is difficult to acquire with sufficient replication under field conditions. Instead, chlorophyll fluorescence (F_v/F_m) and predawn water potential (Ψ_{pd}) are often used in arid and semi-arid ecosystems.

2. F_v/F_m reflects the photoactivation status of photosystem II (PSII), whereas Ψ_{pd} indicates water availability in the rhizosphere. Here we compare these indices with A in two perennial C_4 grasses (native *Heteropogon contortus* and invasive *Eragrostis lehmanniana*) and in seedlings of the C_3 shrub *Prosopis velutina* growing on highly contrasting sandy loam and loamy clay soils in experimental plots. Measurements were made the day prior to and up to 7 days following a 39-mm rainfall pulse after 2 months of drought.

3. A was more sensitive across a broad range of environmental conditions, whereas F_v/F_m and Ψ_{pd} only responded to periods of protracted drought. The use of these measures was further complicated because their values varied daily and we observed different time-lags in their response to precipitation pulses.

4. We suggest sampling schemes and *a priori* measurements to capture the value that is representative for the question of interest, and that match the pulsed biological activity in these ecosystems. Finally, we suggest the use of these measures in combination with measurements providing integration over longer time periods, such as $\delta^{13}C$, $\delta^{18}O$ and N concentration in bulk leaf tissue.

Key-words: chlorophyll fluorescence, photosynthesis, predawn water potential, Santa Rita Experimental Range, semi-arid ecosystems

Introduction

Biological activity in water-limited ecosystems is driven by intermittent ‘pulses’ of water availability. Ecological studies in these ecosystems can be broadly classified as either ‘pulse studies’ or ‘growing season studies’. The main goal of the former is to understand the dynamic response of the system to precipitation pulses (Huxman *et al.* 2004). The latter aims at understanding how seasonal changes in resource availability impact plant community dynamics and processes, such as plant interactions or growth and survival under different environments.

There is an increasing trend to link community dynamics and processes with their underlying physiological mechanisms (McGill *et al.* 2006). The first step towards this mechanistic approach relies on understanding how different environmental conditions lead to different levels of ‘stress’, that is, reduced rates of growth or reproduction (Grime 1989). Because reductions in photosynthetic rates (A) closely follow reduced growth in response to water scarcity (Fitter & Hay 2002), we hypothesized that the former could be an appropriate measure of plant stress. Moreover, A can be mechanistically related to growth (Smith & Stitt 2007) and fitness (Caruso, Maherali & Sherrard 2006), but it is only seldom measured within the context of broad population or community-level growing season

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studies. We evaluated how researchers assess stress in growing-season studies by examining the references cited in a recent meta-analysis on plant interactions in semi-arid environments (Maestre, Valladares & Reynolds 2005), and found that 41 of 56 articles evaluated plant stress through: (i) dark-acclimated values of maximum quantum yield of photosystem II (PSII; F_v/F_m); (ii) predawn water potential (Ψ_{pd}); or (iii) a related variable such as soil water potential (Ψ_s). F_v/F_m reflects the maximal light harvesting capacity of PSII (Demmig-Adams & Adams 2006). Values below their optimal range (0.83 in most plant species, relative units) indicate photoinhibitory stress in response to high or low temperatures, excess irradiance or water stress (Maxwell & Johnson 2000). Ψ_{pd} is an integrated measure of Ψ_s in the rhizosphere, given the assumption that plant water comes into equilibrium with soil water during night (Davis & Mooney 1986). Ψ_{pd} can be linked to drought or freezing-induced mortality, because it is related to failure of vascular supply (Pockman & Sperry 1997; Sperry *et al.* 1998).

It is noteworthy that broad studies on vegetation dynamics provide either Ψ_{pd} or F_v/F_m as the only measures of physiological function, because they reflect rather specific processes. Instead, A encompasses a wider range of physiological processes, and is considered an integrated indicator of the biological activity (Lake 2004), but it is not commonly measured.

All but three studies reviewed by Maestre *et al.* (2005) measured Ψ_{pd} and F_v/F_m at pre-defined regular intervals over the growing season, independent of rainfall events. However, the dynamics of A , Ψ_{pd} and F_v/F_m in arid and semi-arid ecosystems are strongly influenced by the magnitude of water stress prior to new moisture inputs, such that their values vary greatly and independently from each other over very short time-scales following a precipitation pulse (Huxman *et al.* 2004).

In this study we measured A , Ψ_{pd} and F_v/F_m the day before and up to 7 days after a precipitation pulse across functional groups (two C_4 grass species and two cohorts of seedlings of a C_3 woody legume) and contrasting growing conditions (on sandy loam and clay loam soils). Our first objective was to compare Ψ_{pd} and F_v/F_m with A as measures of stress for studies on vegetation dynamics in pulse-driven ecosystems. The second was to analyse the effect that the poor temporal integration of these measurements has on their interpretation. Finally, we provide sampling recommendations.

Methods

STUDY SITE AND PLANT SPECIES

This experiment was performed in two sets of three rain-out shelters on sandy loam and clay loam soils in the Sonoran Desert, at the Santa Rita Experimental Range in southeastern Arizona, USA (see English *et al.* 2005 for a detailed description of shelter location, set-up and protocols). Annual mean precipitation at the site is around 400 mm with 60% falling in the summer and 40% during winter. We evaluated short-term changes in F_v/F_m , Ψ_{pd} and A before and after a 39-mm irrigation pulse under these shelters. Irrigation water was applied on 10 June 2005, after a 2-month drought, and was repeated on 10 June 2006. Measurements for each year were made

the day prior to the watering, and on days 1, 3 and 5 afterwards, and additionally on day 7 in 2006.

The species studied were the C_4 invasive grass *Eragrostis lehmanniana* Ness., the C_4 native grass *Heteropogon contortus* (L.) Beauv., and 1 and 3- to 4-year-old seedlings of the C_3 woody shrub *Prosopis velutina* Woot. Each plot was dominated by one grass species or by *P. velutina* seedlings.

PHYSIOLOGICAL MEASUREMENTS

F_v/F_m was measured in the grasses and seedlings ($n = 3$ for each grass species and woody seedling cohort) with a portable pulse-modulated fluorometer (FMS2, Hansatech Instruments, Kings Lynne, UK) at predawn using procedures described by Maxwell & Johnson (2000). Ψ_{pd} was measured with Peltier thermocouple psychrometers (PST-55 Wescor Inc., Logan, UT) for the 1-year-old *P. velutina* seedlings and with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR) for the 3- to 4-year-old seedlings and both grasses ($n = 3-5$ for each grass and woody seedling cohort). The use of thermocouple psychrometry was necessary to minimize the impact on 1-year-old seedlings. This method required to maintain the leaves at 25 °C and isolated within a sealed chamber, and measurements were made following the manufacturer's instructions. The two different techniques to measure Ψ_{pd} were compared in an independent test by measuring Ψ_{pd} in 24 *P. velutina* seedlings with both methods. We failed to detect any differences in Ψ_{pd} due to instrumentation (*t*-test, $n = 24$, $P > 0.13$).

To compare A responses between species with different photosynthetic capacities (e.g.: C_4 grasses and C_3 woody shrubs), we report photosynthesis as the percent loss of mean maximum assimilation rate (PLA):

$$PLA(\%) = 100 \left[1 - \left(\frac{\hat{A}_{s,d}}{\hat{A}_{s,max}} \right) \right] \quad \text{eqn 1}$$

where $\hat{A}_{s,d}$ is the average ($n = 3-5$) of the A value for species s on day d after the irrigation, and $\hat{A}_{s,max}$ is the maximum of the mean assimilation rates under optimum conditions (during the peak of the rainy season, around 20 mol m⁻² s⁻¹ for *P. velutina* and 30 mol m⁻² s⁻¹ for the C_4 grasses) for species s . Assimilation rates were measured at time of maximum assimilation (07.30 h) with a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE). Levels of light, temperature, leaf-to-air vapour pressure deficit and CO₂ inside the cuvette were set to match environmental conditions (700 mol m⁻² s⁻¹, 30 °C, 3–4 kPa, and 400 p.p.m., respectively).

Gas exchange and chlorophyll fluorescence (F_v/F_m) measurements were conducted on the youngest fully-expanded sun-lit leaves. *Prosopis velutina* leaves were marked and the same leaf was monitored each day during the pulse period. Leaves for measurements on the grasses were randomly chosen on each day of the irrigation-pulse period.

The statistical relationships of PLA with F_v/F_m and Ψ_{pd} were determined through linear regression using SIGMAPLOT 8.0 (SYSTAT Software Inc., San Jose, CA).

Results and discussion

F_v/F_m COMPARED TO A

F_v/F_m values were constant around the optimum 0.83 across species and soil textures, except when PLA rose above 75% (Fig. 1a,b), which occurred mainly on the sandy loam surface. Based on this, we parameterized the following statistical model:

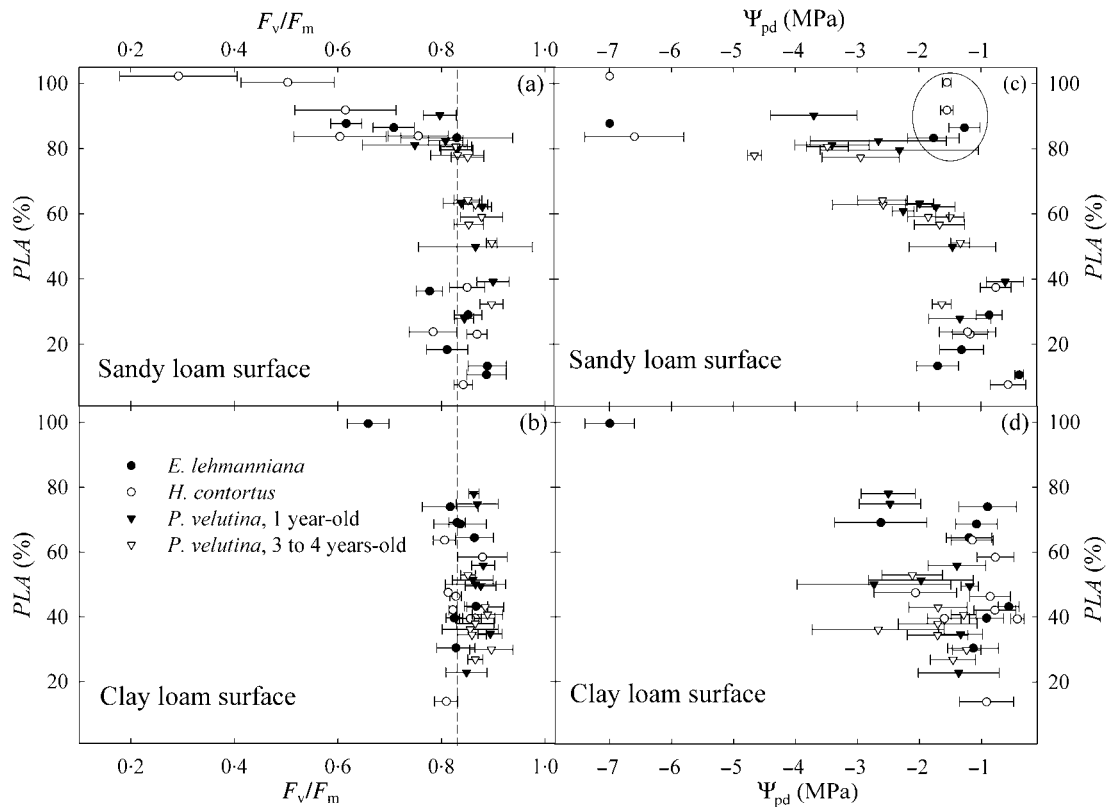


Fig. 1. Relationship between percent loss of assimilation (PLA) and dark-acclimated values of maximum quantum yield of photosystem II (F_v/F_m) (a and b) or predawn water potential (Ψ_{pd}) (c and d) on different soil textures. Each point represents an average value ($n = 3-5$) for each pulse day ($-1, 1, 3, 5$ and 7) for each measured year (2005 and 2006), and species. Error bars indicate standard errors. The dashed line across (a) and (b) indicates the ‘optimum’ value of F_v/F_m (Maxwell & Johnson 2000). Species names and the ellipse on (c) are explained in the text.

$$PLA = \begin{cases} -41.9(F_v/F_m) + 115.6 & \text{if } PLA \geq 75\% (P < 0.0001, r^2 = 0.77) \\ \text{is independent of } F_v/F_m & \text{if } PLA < 75\% (P > 0.5) \end{cases}$$

eqn 2

PLA values $> 75\%$ correspond to the day prior to the pulse and up to 1 day after in *E. lehmanniana* and both seedling cohorts, and up to 3 days afterwards in *H. contortus*. F_v/F_m is typically restored overnight, and it only remained low overnight after protracted drought. Hence, F_v/F_m values indicated PSII inactivation only at extremely high PLA values (Fig. 1a,b).

Ψ_{pd} COMPARED TO A

The sensitivity of Ψ_{pd} was also lower than that of PLA , particularly on the sandy loam surface (Fig. 1c), where Ψ_{pd} remained constant around -1 MPa until PLA increased $> 50\%$:

$$PLA = \begin{cases} -6.6\Psi_{pd} + 53.0 & \text{if } PLA \geq 50\% (r^2 = 0.6, P < 0.01) \\ \text{is independent of } \Psi_{pd} & \text{if } PLA < 50\% (P < 0.15) \end{cases}$$

eqn 3

Ψ_{pd} on the clay loam surface (Fig. 1d) showed higher variation than on the sandy loam surface, and was relatively more decoupled from PLA . Indeed, although regression analyses were statistically significant ($P < 0.05$), there was a large variability not explained by the model ($r^2 = 0.3$).

These results suggest a differential regulation of photosynthesis across soil textures: the linear relationship between PLA and Ψ_{pd} on the sandy loam surface when $PLA > 50\%$ (Fig. 1c), suggests that A was, in this case, mainly controlled by hydraulic limitations. The decoupling between PLA and Ψ_{pd} on the clay loam surface, at any given PLA , and on the sandy loam surface, when PLA dropped $< 50\%$, suggest that non-stomatal limitations exerted then the main control over A (Lambers *et al.* 1998). These results support those obtained by measuring the response of A to increasing CO_2 concentrations (A/C_i curves; Long & Bernacchi 2003) conducted concomitantly in an independent study at the same site (Victor Resco, unpublished data).

Interestingly, the relationships of PLA with F_v/F_m and Ψ_{pd} were independent of the species and mainly influenced by soil type (Fig. 1).

SAMPLING IMPLICATIONS AND TIME INTEGRATION

Deciding what measure to perform ultimately depends on the research question of interest. However, some general considerations should be noted. F_v/F_m provides information on PSII photoinactivation. Our results, in line with other studies (Nippert *et al.* 2007), indicate that F_v/F_m is only sensitive to protracted periods of drought, but it does not realistically capture the seasonal kinetics of plant biological activity in

pulse-driven ecosystems over shorter time-scales (Fig. 1a,b). Ψ_{pd} is a measure of water availability. Our results show that after a threshold value, biological activity becomes independent from water availability (Fig. 1c). We recommend caution in using this index if the researcher does not quantify, *a priori*, the dependency of biological activity on Ψ_{pd} . A is probably a more appropriate indicator of stress for the broad objectives of growing season studies than F_v/F_m and Ψ_{pd} under a wide range of environmental conditions (Fig. 1). A shows a high sensitivity to water scarcity, and even transient declines in A are associated with reduced or impaired growth (Fitter & Hay 2002). Indeed, A has been related to plant growth, survival and reproduction in the field (Caruso *et al.* 2006; Galmés *et al.* 2007), and has the potential to become very valuable in current efforts to understand the mechanistic underpinnings of population and community processes.

The interpretation of A , F_v/F_m and Ψ_{pd} is further complicated because their values may change daily in arid and semi-arid environments characterized by discrete moisture inputs during the growing season (Fig. 2). Instantaneous values and dynamic changes in A , Ψ_{pd} and F_v/F_m depend on patterns and magnitudes of moisture inputs, the duration and magnitude of inter-pulse drying cycles and on differences in drought adaptation among species (Huxman *et al.* 2004). Moreover, they each show a specific time-lag in their response to a moisture input (Fig. 2). For example, Ψ_{pd} is often decoupled from PLA because water infiltration and re-wetting of roots is faster than photosynthetic up-regulation (Huxman *et al.* 2004). Indeed, on the first day after the water pulse (values inside the ellipse in Fig. 1c), we observed an extremely high PLA for both grasses, when Ψ_{pd} was as high as -1.2 MPa. This indicates a time-lag in the response of A to the moisture input following the prolonged drought period. This was supported by the low F_v/F_m values in these plants at this time (Fig. 1a). Hence, growing season studies would benefit from a sampling design that appropriately captures this variability.

Sampling regimes will differ markedly among studies with different objectives. However, some general recommendations are offered. First, we do not recommend the general use of Ψ_{pd} , without a previous field test, like the one reported here, of its relationship with biologically meaningful processes following precipitation pulses. F_v/F_m is most appropriate when the researcher is mainly concerned with treatments that cause a strong reduction in physiological performance, such as 80% PLA . Alternatively, A is more sensitive to environmental changes and we recommend its use across a broader range of conditions. Whenever possible, the combined use of these three measurements (A , F_v/F_m and Ψ_{pd}) is recommended. They depict a rather complete mosaic of plant's physiological performance that could help overcome the temporal limitations of these measurements, as exemplified in the discussion regarding Fig. 1(a,c) in the previous paragraph. Second, it is important to determine under what conditions and at what critical periods during the growing season these proxies will provide relevant information for growing season studies. More specifically, sampling should be timed according to the precipitation pulse regime, instead of following pre-defined or

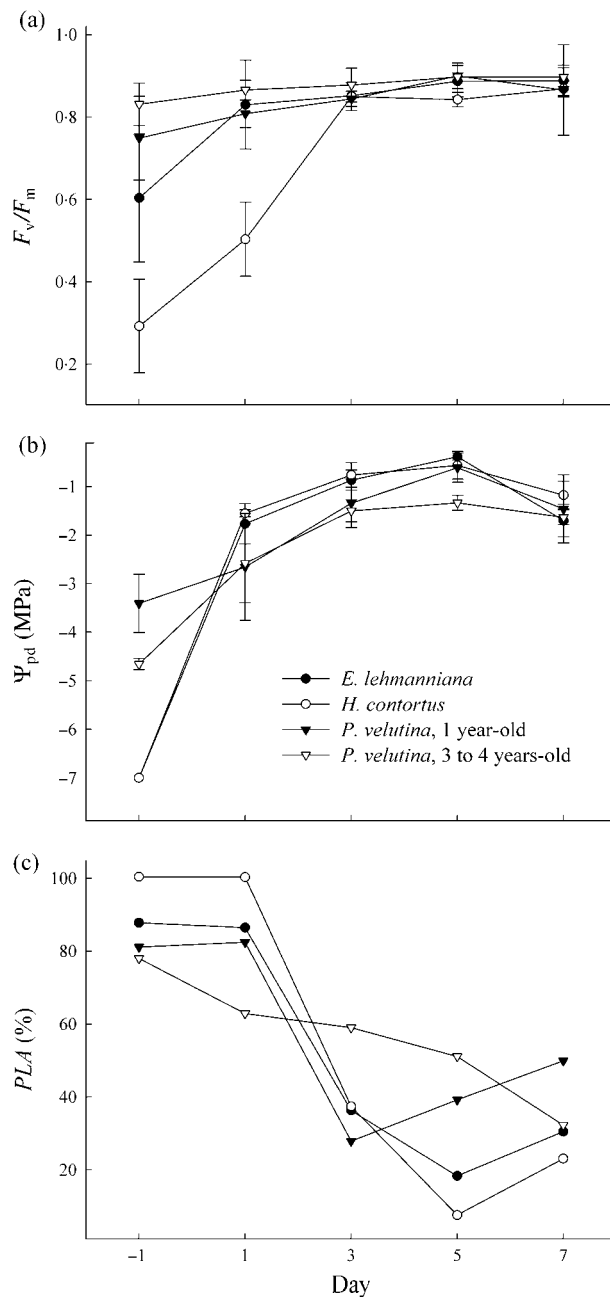


Fig. 2. Representative time response of F_v/F_m , Ψ_{pd} and PLA (a, b and c, respectively) to a 39-mm irrigation pulse applied after a period of drought. Results show the poor temporal integration of these measurements, and the need for a sampling design that adequately captures this dynamic response. Error bars indicate standard errors.

regular sampling intervals. Finally, because values for A , F_v/F_m and Ψ_{pd} are very dynamic during a precipitation pulse (Fig. 2), it is important to capture the whole pulse response in different parts of the growing season. Alternatively, some *a priori* experiments to characterize the pulse response of the species of interest would be of help to identify what days during a precipitation pulse are representative for the question of interest.

COMPLEMENTARY MEASURES

To avoid intensive field sampling, longer term integrators of photosynthetic performance may be more suitable for growing-season studies. Measurements of leaf N concentrations, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values have the potential to partition the different limitations to photosynthesis while integrating long-term responses (Cernusak *et al.* 2007; Farquhar *et al.* 2007; Grams *et al.* 2007). However, their application is not straightforward and knowledge of physiological processes causing variation in the isotope composition and nitrogen content of leaves is also necessary. We therefore recommend the combined use of A along with longer time integrators to better understand the mechanisms behind plant interactions, growth and survival.

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