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Ecophysiological diversity of wild *Coffea arabica* populations in Ethiopia: Drought adaptation mechanisms

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ABSTRACT

Drought is a wide-spread limiting factor in coffee (*Coffea arabica* L.) production, but the development of adapted cultivars is hampered by a limited understanding of the physiological resistance mechanism and the extreme narrow genetic base of plant material used in breeding. Therefore, the objective of this study was to evaluate the ecophysiological diversity of coffees wild progenitors and their potential for the improvement of drought resistance at its primary gene pool and center of genetic diversity, in Ethiopia.

Complementary field and common-garden studies of populations native to habitats spanning a wide range of climatic conditions were examined by combining seasonal measurements in naturally regenerated stands of wild coffee (*in-situ*) with an artificial drought stress experiment (*ex-situ*), where seedlings of these populations were raised under controlled environmental conditions and subjected to different watering and light intensity regimes. Plant ecophysiological behavior was assessed in terms of gas exchange activity, leaf water status determined by water potential and osmotic potential measurements, chlorophyll fluorescence and carbon isotope discrimination.

The results of both experiments revealed that the ecophysiology of wild *C. arabica* populations was strongly influenced by conditions of water deficit, heat and high irradiance, which had combined effects on the plants during drought periods.

However, the coffee plants were able to adapt to the natural and experimental drought conditions; thereby their resistance mechanism was mainly based on the capability to avoid tissue desiccation. In particular, physiological responses were attributed to fine control of stomatal conductance reducing water loss, high rates of photosynthesis and improved water-use efficiency. Overnight re-saturation of tissue water status with no active osmotic adjustment ability was another important characteristic in drought adaptation of the plants.

In addition, the *in-situ* ecophysiological behavior of populations from different habitats revealed contrasting strategies in response to drought stress, thereby this habitat-specific variability was also found under common-garden conditions. Thus, the results of this study provide evidence that there is a genetic basis of the marked ecophysiological diversity in wild *Coffea arabica* and that the contrasting habitat conditions result into different selection pressures for traits related to water-use and promote regional differentiation in adaptation to drought stress.

Furthermore, the coffee plants exhibited substantial phenotypic plasticity for all ecophysiological traits in response to varying soil moisture conditions; thereby the magnitude of plasticity differed markedly among the populations. In particular, plants originating from drier, variable climates showed the highest amount of plasticity suggesting fast adaptation to changing environmental conditions. A high correlation of plastic responses in most characters was an indicator for a high degree of phenotypic integration demonstrating the need for integrative approaches for the evaluation of drought adaptation in this species.

Overall, the profound amount of ecophysiological diversity in the primary gene pool of *C. arabica* found in this study emphasizes its importance as a unique and valuable genetic resource. The specific adaptations to drought stress suggest a high potential for breeding of improved cultivars and stresses the need for conservation of the profound ecophysiological diversity. In particular, the findings suggest an approach of *in-situ* conservation within the evolutionary dynamic ecosystems of the natural habitat of *Coffea arabica* in order to allow the preservation of genes for stress resistance as they co-evolve with their changing environment.

Ökophysiologische Diversität in Wildpopulationen von *Coffea arabica* in Äthiopien: Mechanismen der Trockenstreßadaption

KURZFASSUNG

Trockenstress gehört weltweit zu den Umweltfaktoren, die im erheblichen Maße das Ertragspotenzial von Arabica-Kaffee (*Coffea arabica* L.) beeinträchtigen. Da jedoch die Züchtung stressadaptierter Sorten durch mangelnde Kenntnisse des Resistenzmechanismus sowie der engen genetischen Basis moderner Kulturpflanzen stark limitiert ist, untersucht die vorliegende Arbeit das Ausmaß der ökophysiologischen Vielfalt und Züchtungspotenzial von Arabica-Kaffee in Äthiopien, seinem Ursprung und genetischem Zentrum.

Hierzu wurden vier Fragmente des afromontanen Regenwaldes mit natürlichem Vorkommen von Arabicakaffee entlang eines Klimagradienten ausgewählt und das ökophysiologische Verhalten der Pflanzen während natürlich vorkommender Trockenperioden am natürlichen Standort (*in-situ*) sowie unter kontrollierten Trockenstressbedingungen (*ex-situ*) untersucht. Die Charakterisierung des pflanzlichen Wasserhaushaltes erfolgte durch Messungen des Gaswechsels, der Chlorophyll-Fluoreszenz, des Blattwasserstatus sowie der δ^{13}C Analyse.

Sowohl unter natürlichen als auch unter experimentellen Trockenstressbedingungen war das ökophysiologische Verhalten der Kaffeepflanzen stark von einer Kombination verschiedener Umweltfaktoren wie Bodenwassergehalt, Temperatur, Lichtintensität und dem atmosphärischen Wasserdampfdruckdefizit beeinflusst.

An der Ausprägung der Trockenstressresistenz zeigten sich eine Vielzahl von physiologischen Merkmalen beteiligt, die vornehmlich auf Mechanismen zur Vermeidung der Austrocknung des Pflanzengewebes beruhten, wobei eine mögliche Austrocknungstoleranz durch osmotische Anpassung nicht nachgewiesen werden konnte. Eine Anpassung an Trockenheit erreichten die Pflanzen maßgeblich durch das Aufrechterhalten einer hohen Nettophotosyntheserate, die effektive stomatäre Kontrolle der Transpirationsverluste sowie eine hohe Wasserausnutzungseffizienz.

Weiterhin zeigten die Ergebnisse, dass sich die Kaffeepopulationen hinsichtlich der Anpassung an Trockenstress unterschieden, wobei die Ausprägung unter *ex-situ* Bedingungen auf eine genetische Fixierung der populationsspezifischen Adapationsmechanismen hinweist. Da sich die Unterschiede darüber hinaus als Anpassung an die jeweiligen Umweltbedingungen am natürlichen Standort interpretieren ließen, kann den spezifischen Habitatbedingungen als Selektionsfaktoren eine bedeutende Rolle hinsichtlich der ökophysiologischen Differenzierung der Populationen beigemessen werden.

Weiterhin stellt die Arbeit die phänotypische Plastizität als eine wichtige Anpassungsstrategie in den äthiopischen Kaffeepopulationen an veränderte Umweltbedingungen heraus, hinsichtlich dieser auch Populationsunterschiede nachgewiesen werden konnten. Darüber hinaus verdeutlichten die Ergebnisse die Notwendigkeit einer integrierten Betrachtungsweise der untersuchten ökophysiologischen Merkmale, die in Ihrem Zusammenwirken maßgeblich die erfolgreiche Adaptation an Trockenstress beeinflussen.

Die in dieser Studie nachgewiesene einmalige ökophysiologische Vielfalt der äthiopischen Wildkaffeepopulationen belegt ihren potenziellen Nutzen in Züchtungsprogrammen und macht somit die Erarbeitung nachhaltiger Schutzkonzepte für den Erhalt dieser wertvollen pflanzengenetischen Ressourcen notwendig. Hierbei sollten Strategien einer *In-situ*-Konservierung im Vordergrund stehen, um so den Erhalt und die kontinuierliche Weiterentwicklung der ökophysiologischen Diversität von *C. arabica* für heutige und auch zukünftige Nutzungsinteressen gewährleisten zu können.

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ABBREVIATIONS

a.s.l.	above sea level	m
A_{max}	maximum photosynthetic rate	$\mu mol\ m^{-2}\ s^{-1}$
A_{net}	net photosynthetic rate	$\mu mol\ m^{-2}\ s^{-1}$
ANOVA	analysis of variance	
c_a	partial pressure of ambient CO_2	
c_i	partial pressure of intercellular CO_2	$\mu mol\ mol^{-1}$
CV	coefficient of variation	%
E	transpiration	$mmol\ m^{-2}\ s^{-1}$
F_m	minimum fluorescence	
F_o	initial fluorescence	
F_v	variable fluorescence	
F_v/F_m	PS II efficiency, yield	
g_s	stomatal conductance	$mol\ m^{-2}\ s^{-1}$
IC	induction curves	
kg	kilogram	
LAI	leaf area index	
m	meter	
max	maximum	
md	midday	
meq	milliequivalent	
min	minimum	
mm	millimeter	
MPa	MegaPascal	
n.s.	not significant	
PAR	Photosynthetically active radiation	μmol photons $m^{-2}\ s^{-1}$
pd	predawn	
PPFD	photosynthetic photon flux density	μmol photons $m^{-2}\ s^{-1}$
ppm	parts per million	
$P_{predawn}$	predawn turgor potential	MPa
PS II	photosystem II	
r-ETR	relative electron transport rate	
RLC	rapid light curves	
RWC	relative leaf water content	%
S	drought susceptible index	
SD	standard deviation	

SMC	soil moisture content	%
T_{air}	air temperature	°C
T_{leaf}	leaf temperature	°C
VPD	vapor pressure deficit	kPa
WUE_i	instantaneous water-use efficiency (A/E)	μmol mmol^{-1}
$\Delta \Psi$	diurnal change in leaf water potential	MPa
$\delta^{13}C$	carbon 13 isotope discrimination	‰
π_{md}	midday osmotic potential	MPa
π_{pd}	predawn osmotic potential	MPa
Φ_{PSII}	variable quantum yield of PS II photochemistry	
Ψ_{md}	midday leaf water potential	MPa
Ψ_{pd}	predawn leaf water potential	MPa

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1 INTRODUCTION

1.1 Background and problem statement

With environments shifting dramatically in climatic conditions in the last decades, drought has become a serious constraint on *Coffea arabica* production worldwide with dramatic impacts on producers' incomes and coffee production viability (DaMatta and Ramalho 2006). Specifically, small-scale producers in developing countries play a major role in coffee production; they lack irrigation facilities and therefore constitute the most vulnerable group (DaMatta and Ramalho 2006). Due to the predictions of global climatic change with its impacts on temperature, moisture regimes and the occurrence of extreme weather events (IPCC 2007), it is expected that this situation will deteriorate in the near future (Schulze, Kelliher et al. 1994; Sala 2000; Peuke, Schraml et al. 2002).

In this context, developing crop plants with inbuilt drought resistance mechanisms is considered a promising strategy for sustainable economic production in drought-prone environments and is therefore an important goal for breeders targeting these more marginal areas. Until now, considerable research on the ecophysiology of Arabica coffee has been undertaken (Rena, Barros et al. 1994; Barros, Maestri et al. 1995; Carr 2001; Maestri, Barros et al. 2001). Nevertheless, conventional breeding has met drought adaptation with only little success due to limited knowledge of plant stress physiology. Difficulties arise because breeding programs do not account for the complex multidimensional stress character of drought in field-grown plants where a range of different environmental influences prevent the cultivars from expressing their full genetic potential (Medrano, Chaves et al. 1998). Hence, plants selected in breeding programs may not necessarily bring the desired performance when transferred to other environments, because their susceptibility varies depending on stress severity and duration as well as on the ontogenetic stage of the plant (Lorens, Bennett et al. 1987). Another important constraint with regard to the genetic improvement originates from the fact that major emphasis in breeding has been placed on domesticated cultivars of *C. arabica,* which has been shown to have only a limited potential for the breeding of new varieties (Meyer 1965; Tanksley and McCouch 1997; Hein and Gatzweiler 2006). In fact, there is low genetic diversity in modern cultivars of *C. arabica* (Baruah, Hendre et al. 2003), mainly due to the limited number of original plants from which the main crop plants were derived (Tewolde 1990; Teketay 1999). Additionally, early domestication, artificial selection and intensive breeding severely eroded allelic variations resulting in a narrowed genetic base of coffee cultivars, which renders them more susceptible to environmental stresses (Meyer 1965; Tanksley and McCouch 1997; Tadesse, Denich et al. 2002; Hein and Gatzweiler 2006).

Due to the low level of genetic diversity of modern cultivars of *C. arabica*, interest is growing in the wild relatives of this species, which can be found in Ethiopia, its primary center of origin and center of genetic diversity (FAO 1968). The wild populations occurring there are believed to show high diversity that can be used as a valuable raw material from which breeders can craft more productive varieties to meet the needs of world-wide efforts to improve the productivity, resilience and quality in cultivated Arabica coffee (Hein and Gatzweiler 2006). Especially with regard to enhancement of drought adaptability, a high potential can be expected, since wild *C. arabica* populations occupy a broad habitat range, above all characterized by the spatio-temporal variation in water availability. Due to this predominant feature and the tight interrelation with plant ecophysiological behavior (Blum 1988), the contrasting environmental conditions of the distant habitats are likely to create distinct selection pressures related to water-use, which promotes the diversification of ecophysiological traits among the wild coffee populations (Parsons 1988; Sandquist and Ehleringer 1998). However, it is unclear, if the mechanisms, which allow wild *Coffea arabia* to successfully occupy this broad habitat range with contrasting environmental conditions, results from patterns of phenotypic plasticity of genetically similar populations or from the subdivision of genetically distinct ecotypes for each habitat type, which both represent alternative means of adaptation (Bradshaw and Hardwick 1989; Schlichting and Smith 2002).

Though these benefits of biodiversity are obvious it still remains largely unknown how much useful ecophysiological diversity exists in wild coffee progenitors and to date there has been no sufficient evaluation of their potential value. In fact, management of the last remaining wild progenitors of *C. arabica* is rather poor, and anthropogenic activities such as deforestation and land-use changes (Gole 2002) are among the main reasons why the Afromontane rainforests, which provide these highly valuable natural resources, are disappearing at an alarming rate. In addition, Ethiopian coffee farmers tend to replace wild coffee plants by improved and uniform varieties. As a consequence, there is a permanent loss of diversity of Arabica coffee (Solbrig 1992), and though some efforts to preserve the wild relatives of this species have been made, the destruction of their natural habitats is still ongoing. Consequently, urgent action has is necessary to manage the finite genetic resources in a sustainable way. With growing awareness of the irreversible loss of plant genetic resources, conservation strategies are obligatory if the remaining wild populations of Arabica coffee are to continue to serve as the principal source of sustenance to meet current and future unforeseen needs.

In order to stop the ongoing process of habitat destruction and the parallel loss of wild *C. arabica*, there is consequently a need for research focusing on the value and functional importance of these wild genetic resources and the ecological implications of their loss will help to put priority on conservation strategies in decision-making processes. Doing so, a comprehensive exploration of the extent of ecophysiological diversity found in the genetic resources of *C. arabica* as well as an in-depth understanding of their adaptive mechanisms and response to drought episodes is obligatory. Especially, the examination of the mechanism that allows wild *C. arabica* to occupy its broad habitat range will also contribute to breeding efforts by providing insights into possible strategies for improving drought resistance in coffee production. Moreover, it allows the identification of drought resistant coffee populations, helps to assign conservation areas of existing diversity, and provides recommendations for optimal concepts of conservation of the last and endangered wild populations of *C. arabica.*

1.2 Research objectives

To address this need for information on the ecophysiological diversity of *C. arabica*, natural wild populations from habitats with contrasting climatic conditions were studied. Following the overall hypothesis, the environmental gradient among the study sites was likely to promote regional differentiation in ecophysiological traits. Hence, the expectation of varying drought response may possibly allow the identification of drought-adapted coffee populations. However, due to the fact that gradients in landscapes generally vary in more than one property and traits are differently influenced by genetics and environment, measurements on field-grown plants (*in-situ* study) were combined with a study under semi-controlled environmental conditions in an *ex-situ* experiment, thereby separating the effects of water availability, irradiance and heat stress in order to provide a comprehensive screening of the wild *C. arabica* populations.

More specifically, the objectives of this study were:

1. To evaluate the dependency of ecophysiological behavior of wild *C. arabica* on environmental conditions
2. To determine physiological traits that contribute to the drought-resistance mechanism in wild *C. arabica*
3. To assess the degree of ecotypic differentiation in ecophysiological behavior in wild *C. arabica* in relation to the environmental conditions of their natural habitat
4. To understand whether phenotypic plasticity is a strategy used by wild *C. arabica* in order to survive variable habitat conditions

The findings are used to predict plants performance under varying environments, develop breeding strategies as well to assign suitable conservation areas and develop criteria for a successful conservation strategy.

1.3 Thesis outline

This thesis is structured into six chapters. Chapter 1 introduces the problem of drought stress as a serious threat to production of *C. arabica*, the necessity of research on ecophysiology in wild coffee populations and the research objective of the thesis. Chapter 2 reviews the theoretical framework on drought resistance in Arabica coffee and the need to focus on wild provenances at the center for genetic diversity in Ethiopia, while Chapter 3 gives an overview of the material and methods used in the study. The results of the study are presented in Chapter 4, which examines the habitat contrasts of the natural stands of *C. arabica* and characterizes seasonal differences in the ecophysiological behavior of field-grown *C. arabica* populations and their differences in drought resistance at the seedling stage raised under common environmental conditions. The major experimental findings are discussed in Chapter 5, which are finally used to develop a breeding concept for drought resistance as well as to give recommendations for conservation strategies for the wild *C. arabica* populations of Ethiopia (Chapter 6).

2 LITERATURE REVIEW

2.1 Influence of drought stress on the production of *Coffea arabica*

Coffee is one of the most important commodities in international agricultural trade and its production forms the economic backbone of many countries worldwide (FAO 2003).With its significance as a source of export income, the area of agricultural land it occupies and the number of people employed in this sector, coffee plays a distinctive role in these societies. From about 90 *Coffea* species, *Coffea arabica* L. (Arabica coffee) is the most valuable in world trade, contributing to over 70 % of the world coffee production (Willson 1999). Coffee is confined to the intertropical zone (Smith 1989), where it grows as a shade-adapted bush or small tree at altitudes between 1,000 and 2,000 m a.s.l. with an optimum mean annual temperature range of 18 to 21°C (Alègre 1959). Due to its requirements with respect to amount and seasonality of precipitation, coffee grows in regions where rainfall is 1500 to 1800 mm yr^{-1} with a dry spell corresponding to the dormant period that proceeds the main flowering period.

In the last decades, however, the coffee cultivation area has been forced into environments unsuitable for its production, with profound impacts on its growth and productivity (DaMatta and Ramalho 2006). The main reason is considerable land-use changes. First, on farm land that has traditionally been used for coffee production, farmers tend to replace coffee plants by other more profitable cash crops in order to counteract the reduced profits due to the collapse of world coffee prices (Fritsch 2002), so that coffee is shifting to environments with less favorable growing conditions. Secondly, while early plantations under shade simulated the natural habitat of coffee accounting for the fact that it has evolved as an understory shrub showing all physiological and structural characteristics of a shade adapted plant, the practice of shading as a regular cultural practice is being increasingly abandoned. Clearly, traditional production systems are being more and more converted into industrial plantation models of sun-grown coffee due to the higher yield potential there (Malavolta 2000). Thirdly, changes driven by a combination of elevated temperature and an increased occurrence of water deficits by altered rainfall patterns (Easterling, Meehl et al. 2000; IPCC 2007), as predicted in climate-change scenarios, are expected to enhance the vulnerability to drought in these areas (Chaves, Pereira et al. 2003). This includes reduced total quantities of rainfall and an altered temporal distribution, which results from a combination of increased intensity of convective rainfall events and longer dry intervals separating these, leading to lower overall soil moisture (Fay, Carlisle et al. 2002). Cumulatively, these reasons result into less favorable growthing conditions for coffee production, thereby drought stress will steadily become one of the most important environmental stresses affecting coffee production worldwide (DaMatta 2004; DaMatta and Ramalho 2006).

In general, drought stress may be induced by multiple environmental variables comprising high temperatures, solar radiation, saturated vapor pressure deficit, low rainfall amounts, a poor precipitation distribution as well as increasing intensities of soil moisture deficits (DaMatta 2003). In water-deficit environments, plants may experience reduced soil moisture availability as well as atmospheric drought. Low and erratic precipitation results in different patterns of soil moisture recharge and reduced water-uptake by the plants (Reynolds, Kemp et al. 2004). However, plants may also be affected by atmospheric drought conditions, thereby changes in the evaporative demand of the atmosphere through a higher vapor pressure deficit of the surrounding air during dry periods increases the transpirational demand and hence the water loss of plant tissues to the atmosphere. Besides water availability, light is another major environmental factor influencing growth, reproduction and distribution of Arabica coffee during dry periods. Coffee as a shade-adapted plant has a very low saturating irradiance for leaf photosynthesis (Rhizopoulou and Nunes 1981; Fahl, Carelli et al. 1994; Ramalho, Pons et al. 2000) and exposition to high irradiance can seriously impact on plant performance by limiting photosynthesis, resulting in reduced net carbon gain and plant growth (Lambers, Chapin et al. 1998). Plants that are exposed to irradiance as high as 2000 µmol m^{-2} s^{-1} may undergo a period of light-induced inhibition of the light reactions of photosynthesis, which results from the absorption of light energy in excess of the leaf capacity to utilize it for productive electron transport. However, photoinhibition ranges from an irreversible photodamage of photosystem II centers to a protective, readily reversible regulatory mechanism. In fact, coffee plants show a certain ability to acclimate to sunlight (Ramalho, Pons et al. 2000), though this depends on competition for water and nutrients, pest and disease incidence (Beer, Muschler et al. 1998), which implies the need of optimal growing conditions and often high levels of external inputs for sun-grown coffee (Fournier 1988; Muschler 1997). Climate conditions where plants have to cope with an exposure to high irradiances are also often accompanied by high temperatures and each effect may be superimposed by the other. However, with coffee cultivation under open sun becoming a common practice in many parts of the world, coffee is frequently exposed to temperatures of 40°C and more (Maestri, Barros et al. 2001), which is more than double the optimal temperature range for arabica coffee. Thus, overheating of the leaves is a common feature, which may have devastating effects on plant metabolism by disrupting cellular homoeostasis and uncoupling major physiological processes. The results are not only a depressed growth but also an inadequate storage capacity, abortion of flowers, acceleration of development and ripening of fruits, and hence a loss of quality (Cannel 1985).

These stresses occur simultaneously under natural conditions and their combination may have interactive effects on plant development and fitness in a manner that may be different from those caused by each of the different stresses individually. Thus, drought stress has to be seen as a multi-factorial syndrome in which water deficit, heat and radiation stress all interact to reinforce one another.

2.2 Resistance to drought stress in *Coffea arabica*

2.2.1 Ecophysiological traits involved in drought resistance

Plants may exhibit a specific drought resistance mechanism, which refers to the ability of a crop plant to preserve growth and reproduction under drought conditions with minimum loss of fitness under stressful growthing conditions relative to constraint-free environments. Precisely, drought seriously affects all aspects of plant physiology (Stebbins 1952; Bohnert, Nelson et al. 1995; Bray 1997), thus plants have evolved several ecophysiological acclimation strategies in response to drought stress. As a consequence, plant resistance to these environmental constraints has to be seen as a complex phenomenon that results from a serious of integrated events occurring at different levels of plant organization (Taiz and Zeiger 2002).

The closure of stomata, which are pores in the leaves allowing gas exchange of CO_2 and H_2O, is among the earliest physiological responses to drought that protects the plants from extensive water loss during periods of reduced water availability (Slatyer 1967; Tyree and Sperry 1988). As a consequence, plants avoid catastrophic influences from tissue dessication and are able to maintain a favorable hydraulic status of their tissues above a critical threshold, which allows them to remain metabolic functionable. When water is abundant, stomatal sensitivity has furthermore the ecological advantage of reducing excessive tree water-use, prolonging the period with favorable soil water status. Thereby, stomatal conductance may be controlled by internal plant water status as well as by changes in environmental factors, such as light, vapor pressure deficit and air temperature (Baldocchi, Luxmore et al. 1991; Jones 1992; Hinckley and Braatne 1994).

E.g., plants without an effective water supply system may rely on specific adaptations for controlling water loss by stomatal sensitivity (Kramer 1980). However, stomatal closure can also be directly influenced by soil moisture conditions through chemical signals from root to shoot (Davies, Wilkinson et al. 2002). Especially plants classified as desiccation avoiders, which have dehydration-sensitive tissues, may rely on this non-hydraulic root-to-shoot signaling to avoid transpirational water loss and potential leaf death during drought, whereas plants classified as desiccation toleraters might be less sensitive to non-hydraulic drought signaling because they have tissues better adapted to withstand tissue dehydration.

Because plant photosynthesis is also constrained by excess light co-occurring with heat and water deficit during drought conditions (Tenhunen, Lange et al. 1984; Valladares and Pearcy 1997), the extent to which photosynthetic capability is maintained during periods of drought may play an important role in adaptation to these environments. In general, a decrease in net photosynthesis can be attributed to either a decrease in stomatal conductance and/or non-stomatal limitations (Bethke and Drew 1992). Since stomata influence the influx of CO_2 into the leaves, a reduction in stomatal conductance to conserve water inevitably leads to a decrease in the CO_2 concentration in the intercellular spaces and at a constant demand for CO_2 by the chloroplasts, hence leads to a CO_2 "supply" limitation of photosynthesis by restricting the carboxylation reaction of the enzyme ribulose 1,5-biphosphate carboxylase (RuBisCO) during photosynthesis (Farquhar and Sharkey 1982; Medrano, Escalona et al. 2002). In addition to stomatal limitation, however, plants may also experience an inactivation of photosynthetic reactions due to biochemical limitations (Woodrow and Berry 1988). Specifically, plants may experience excess light energy that cannot be used in photochemistry, due either directly to an increase in irradiance or to a decrease in photosynthesis at a constant irradiance in the presence of other stress factors like drought (Krause and Weiss 1984; Demmig-Adams and Adams 1992; Hendrickson, Ball et al. 2003). This may result in a photooxidative destruction of the photosynthetic apparatus defined as chronic photoinhibition. This is a decline of maximal leaf photochemical efficiency, which is characterized by sustained depression of the photosystem II (PS II) quantum yield (Demmig-Adams and Adams 1992; Osmond 1994). However, plants have developed mechanisms to dispose of this excess excitation energy. Under these circumstances, regulation of light harvesting is necessary to balance the absorption and utilization of light energy, thereby minimizing the potential for photoxidative or photoinhibitory damage. While the majority of the irradiance absorbed by the leaves is either utilized by the PS II photochemical pathway to produce chemical energy (photochemical quenching), it is the capacity of plants to thermally dissipate absorbed light by a nonradiative process (non-photochemical quenching) that plays a central role in leaf photoprotection from net photo-oxidative damage under these environmental stresses (Demmig-Adams and Adams 1992; Ort and Baker 2002). When these protective mechanisms are at work, leaves experience a reversible depression of photochemical efficiency of PS II, termed dynamic photoinhibition (Krause and Weiss 1991; Osmond 1994). However, two different methods allow distinguishing between stomatal and non-stomatal limitations of photosynthesis. Gas exchange measurements provide information about the degree of stomatal constraints on photosynthesis, whereas photodamage to PS II reaction centers can be detected with high sensitivity from changes in chlorophyll fluorescence (Long, Humphries et al. 1994; Maxwell and Johnson 2000; Baker and Rosenqvist 2004).

Stomata are the shared sites of gas exchange regulating water loss and carbon uptake of plants (Cowan 1982). Thus, the parameters of stomatal conductance, transpiration and photosynthesis are highly interrelated with the trade-off between acquiring CO_2 for net photosynthesis and the water lost to transpiration, which determines another important parameter in plant drought resistance mechanisms, i.e. water-use efficiency (Larcher 1995; Fitter and Hay 2002). This characterizes the efficiency at which plants fix CO_2 relative to their rate of H_2O loss and can be defined as either short- or long-term: Instantaneous water-use efficiency (WUE_i) as an short-term indicator of drought resistance by either high net photosynthesis and/or low transpiration can be deduced from porometry measurements, because changes in gas exchange are expected to be important determinants of fitness in drought conditions and can occur within minutes of water deficit. However, there are productivity trade-offs associated with greater WUE, thus, optimal values are expected to differ depending on water availability. In periods with optimal water supply, plants are predicted to make maximal use of water when it is available by fully opening their stomata and assimilating as much carbon as possible, thereby exhibiting low WUE. However, when the water supply is limited, drought-resistant plants should minimize water loss by greatly increasing WUE. Nonetheless, this means that low WUE would be related to low productivity in wet environments on the one hand and a certain stability of coffee yield in drought-prone environments on the other. However, approaches based on instantaneous measures as a selection criterion require frequent sampling and the results are difficult to relate to long-term productivity. However, long-term measurements of physiological performance related to soil or atmospheric drought have been simplified by the discovery of a strong correlation between carbon isotope composition ($\delta^{13}C$) of plant tissues and instantaneous water-use efficiency (Farquhar and Richards 1984; Farquhar, Ehleringer et al. 1989; Meinzer, Goldstein et al. 1990) as well as whole plant water-use efficiency (Hubick, Farquhar et al. 1986). Based on the ratio of the naturally occurring stable isotopes ^{13}C and ^{12}C, this technique provides an integrated measurement of the internal plant physiological and external environmental properties that influence photosynthetic gas exchange over the time during which carbon is fixed (Farquhar, Ehleringer et al. 1989). Generally, plants with high $\delta^{13}C$ are thought to have a high WUE. The method shows a high repeatability and low genotypic by environment interaction, thus making it very attractive as a screening tool in breeding programs (Zhang, Fins et al. 1994). Despite the advantages of the carbon isotope analysis, however, the physiological basis of differences in WUE can not be determined from carbon isotope discrimination measurements alone, because these do not show whether increased water-use efficiency arises from a change in either, or both, stomatal conductance or intrinsic leaf photosynthetic capacity. Hence, carbon isotope discrimination provides an integrated estimate of the “intrinsic water-use efficiency” and therefore complements well instantaneous gas exchange measurements.

Another important adaptive mechanism in plant drought response is the osmotic adjustment ability, which enhances tolerance to desiccation of plant tissues (Turner and Jones 1980; Morgan 1984). It allows leaf hydraulic status to be lowered without an accompanying decrease in leaf turgor and hence turgor-dependent processes such as tissue metabolic activity to continue (Morgan 1984). For example, osmotic adjustment has been shown to facilitate the maintenance of photosynthesis and stomatal conductance at low leaf water potential, to delay leaf senescence as well as to improve yield under water-limited conditions. Specifically, plants can osmotically adjust through an active net accumulation of inorganic and organic solutes in cells in response to decrease in water potential. Hence, the osmotic potential of the cells is lowered, and this acts as a driving force for water uptake and attracts more water into the cell (Cosgrove 1986; Taiz and Zeiger 2002). However, osmotic adjustment may not prevent decreases in growth and gas exchange in response to drought (Jones and Rawson 1979), rather it allows normal physiological activity to continue at reduced rates (Turner and Jones 1980) and helps to maintain tissue integrity and the potential for the rapid resumption of growth when drought stress is relieved. Especially with respect to young seedlings, osmotic adjustment may provide an ecological advantage by maintaining metabolic activity under suboptimal conditions during establishment when roots have not yet reached deep soil water (Lemcoff, Chimenti et al. 1998). Furthermore, the ability to apply mechanisms of osmotic adjustment may be conditioned by the light availability of the specific environment, because the increased solute concentration responsible for osmotic adjustment may have a detrimental effect because of the additional energy requirement. Thus, lower osmotic adjustment ability in leaves grown under low-light environments has been reported, whereas a higher photosynthetic capacity under sunny environments allows an active accumulation of osmolytes (Abrams and Mostoller 1995; Delpérée, Kinet et al. 2003).

2.2.2 Constraints in breeding for drought resistance

Despite considerable progress in the study of abiotic stresses in recent years (Rena, Barros et al. 1994; Barros, Maestri et al. 1995; Maestri, Barros et al. 2001; DaMatta 2004), however, breeding for drought stress resistance in *C. arabica* still remains difficult, and there has been little success. The reasons are multifold. First, difficulties in breeding for drought adaptation are compounded by an incomplete knowledge of the physiological basis of the productivity of *C. arabica* under resource-limited conditions as well as a lack of adequate screening methodologies. This limits the progress in plant improvement programs. In fact, only a few studies account for the multidimensional stress character of drought stress encountered by field-grown plants, whereas the majority of experiments testing the response of plants to changes in environmental conditions focuses on a single stress treatment under controlled environmental conditions. A combination of drought, radiation and heat stress may alter plant metabolism in a manner that may be different from that caused by each of the different stresses individually, thus having a more detrimental effect on the growth and productivity of plants (Craufurd and Peacock 1993; Savin and Nicolas 1996). The response of plants to abiotic stresses in these studies may, therefore, be very different from tests under controlled environments, which do not incorporate a more realistic environmental complexity into their experimental designs. Furthermore, traits determining drought adaptation may be highly interrelated, and a simple and direct link between a particular trait and the maintenance of yield under drought has never been proven (Ludlow and Muchow 1990). Furthermore, the development stage of the crop is another important feature in plant stress response, since plant susceptibility highly depends on the severity and time of the stress in relation to the growth stage of the crop (Lorens, Bennett et al. 1987).

Another difficulty lies in the inheritance and heritability of the drought resistance mechanism. In breeding programs, screening for variation in ecophysiological traits is often conducted under one type of test environments, mostly generated under controlled environmental conditions. Breeding methods for *C. arabica* for example have usually been undertaken under environmental conditions where the crop is grown under stress-free conditions. If the screening environment is identical with the target environment, which can be defined as the set of all environments, field and seasons in which an improved variety is targeted to perform well (Cooper and Somrith 1997), high levels of repeatability and heritability can be observed. However, the concept of expression of maximum genetic potential in sub-optimum condition is debated as a possible genotype-by-environment interaction, as traits related to drought resistance may restrict the high-yielding genotype to perform well under drought and make it difficult to identify consistently superior genotypes. Thus, a key requirement is to know the target environment in which the improved varieties are to be grown and to select the appropriate testing environments that best represent the target environments to which the breeding program is directed. However, breeding under actual drought conditions as an alternative breeding approach suffers from the difficult of predicting environmental conditions. In fact, the intensity of drought can change drastically over space and time; hence it is necessary to understand the expression of these traits in response to the broader range of environments. (Tuberosa and Salvi 2006) conclude that, in environments with varying drought stress, it may be preferable to breed for phenological plasticity to enable genotypes to profit from favorable growth periods. This adaptive plastic response may allow them to maintain function and fitness through rapid, flexible and functionally appropriate response across a range of environments. Furthermore, knowledge on how plant performance depends on environmental variables allows being able to foresee possible changes in the future.

Though some traits related to drought resistance have been identified in *C. arabica*, conventional breeding efforts have been seriously constrained. The main reason is the narrow genetic base of modern cultivars due to the allotetraploid origin and self-fertility of Arabica coffee (Lashermes, Combes et al. 1999; Baruah, Hendre et al. 2003). Moreover, modern coffee varieties go back to only a few plants taken from Ethiopia and have therefore captured only a limited portion of the genetic diversity of their wild progenitors. Further domestication, artificial selection and intensive modern plant breeding severely eroded allelic variations, which has rendered modern crop varieties more vulnerable to environmental stresses (Tanksley and McCouch 1997). Therefore, base broadening or gene pool enrichment is a promising approach. However, it still remains largely unknown how much useful genetic diversity exists in Ethiopia, the center of genetic diversity for Arabica coffee.

2.3 Wild *Coffea arabica* populations in Ethiopia

2.3.1 Ecophysiological diversity and its importance for breeding purposes

In contrast to the narrow gene pool of modern *C. arabica* cultivars, the wild progenitors of this species found in Ethiopia, its place of origin and primary center of genetic diversity (FAO 1968), are believed to be genetically highly diverse. They represent the last remaining resources of genetic variability of *C. arabica* and constitute the building blocks of modern plant breeding, being highly valuable for enriching the genetic basis of cultivated *C. arabica* germplasm (Montagnon and Bouharmont 1996).

In the south-western and south-eastern part of the highlands of the country, wild coffee evolved as an evergreen, self-pollinating shrub in the understory of Afromontane rainforests fragments (Figure 1). There it is largely represented by uniquely adapted and genetically diverse landraces and wild types, which are geographically separated and isolated from each other due to settlement and farming activities (Sylvain 1955; Rhizopoulou and Nunes 1981). Over its area of occurrence, the species shows a broad ecological amplitude occupying a very wide geographic range over which there is extreme environmental variability. It grows within an altitudinal range of 1000 and 2000 m a.s.l with the most suitable range being 1500 – 1800 m a.s.l., on a wide range of soil types, with varying acidity, whereas slightly acid soils, as present under montane forest in southern Ethiopia, are the most suitable (Krug and De Poerck 1968). Air temperature shows little

Figure 1: **Wild *Coffea arabica* in the undergrowth of the Ethiopian Afromontane rainforest**

seasonal variation, averaging annually at about 20°C with an optimum average annual temperature between 18 and 21°C (Alègre 1959). Moreover, wild populations occur under a range of water availability regimes influenced by precipitation and drought longevity over relatively long distances in west-east directions. This range encompasses areas where precipitation varies with an optimum annual rainfall range of 900-2200 mm for arabica coffee (Alègre 1959). Furthermore, rainfall is seasonal in these areas, and natural stands of arabica coffee are subjected to alternating wet and dry seasons. An alternating rainfall pattern is obligatory for the stimulation of flower opening in this species, since coffee undergoes a period of floral dormancy and its anthesis is associated with a dry periods and quiescent wet period. However, the length of the dry season is extremely variable among geographic regions; so that differences in rainfall intensity are accompanied by temporal variability in precipitation and drought conditions. Hence, the habitats of arabica coffee differ strongly from each other with regard to habitat quality; thereby water availability can be accounted for as the most striking micro-geographic distribution pattern.

Due to this broad habitat range, wild *C. arabica* in Ethiopia presumably tolerates great environmental changes in space and in time, which demand constant plant adjustment providing fitness in a variable habitat (Futuyama and Moreno 1988; Van Tienderen 1997). Moreover, water availability is the resource most limiting to ecophysiological behavior and a critical factor in plant performance, thus strong and recurring selective pressure is likely to be imposed on these populations, driving the diversification of physiological traits. Consequently, wild *C. arabica* is likely to exhibit intra-specific differences in ecophysiological behavior (Stebbins 1952; Bohnert, Nelson et al. 1995; Bray 1997). Clearly, this adaptation to a wide range of different habitats (Baazaz 1996) may result from either individual phenotypic plasticity, in which different physiological behaviors are produced by the same populations in different environments, or by genetic differentiation of populations comprising genotypes especially adapted to local environmental conditions (Gregorius and Kleinschmit 1999). In general, populations are expected to differentiate if consistent selection pressures cause directional changes that erode genetic variation and fix population responses, resulting in locally specialized ecotypes. In contrast, selection pressures that vary spatially and temporally in water availability can promote plastic trait responses to environmental conditions in individual plants rather than ecotypic differentiation (Donohue, Pyle et al. 2001). In addition, habitats can differ in their degree of environmental variability as it is true for the wild *C. arabica* populations; where the range and pattern of variation in environmental factors like moisture availability differ consistently between the sites. In such cases, individuals of all populations may differ genetically in the amount and precise pattern of plasticity their individuals (Scheiner and Callahan 1999). Consequently, the pattern of selection on plasticity is expected to differ between habitats, leading to habitat-correlated variation in plasticity.

2.3.2 Need for conservation

Despite their importance and potential commercial value for future demands, the rich genetic resources of wild *C. arabica* face serious threats to their survival. Primarily due to anthropogenic activities, the Ethiopian Afromontane rainforests representing the natural habitats of arabica coffee are diminishing at unprecedented rates, and it is estimated that these forests will have disappeared by 2015 (Tadesse, Demel et al. 2001; Demel 2002). Timber cutting for construction and fuel wood purposes as well as increased land requirements for cultivation of other cash crops is causing a reduction of the forest cover leading into destruction, fragmentation and degradation of these habitats. Additionally, farmers tend to replace wild coffee populations by improved and uniform varieties. In summary, these dramatic manifestations of the poor management of these highly valuable natural resources are resulting in a loss of diversity of arabica coffee (Solbrig 1992). As a consequence, there is an urgent need to manage the finite genetic resources in a sustainable way, and conservation strategies are obligatory if the remaining wild populations of arabica coffee are to continue to serve as principal source of sustenance.

In general, there are two approaches for conservation of plant genetic resources, which should be seen as complementary rather than as substitutes (Nevo 1998). *In-situ* conservation involves maintaining genetic resources in the natural habitats where they occur, while the *ex-situ* approach involves conservation outside the native habitat like seed storage, field gene banks and botanical gardens. Within *ex-situ* strategies, there have been no alternatives for field collections for long-term germplasm conservation in *C. arabica*, because coffee seeds are recalcitrant and with the use of conventional seed storage methods, the coffee seeds will only be viable for a maximum of 3 years (Van der Vossen 1985). In addition, initial failure of *ex-situ* conservation efforts can occur when there is only limited information on the biological and ecological requirements of wild *C. arabica*. Thus, germplasm is lost due to diseases and pests or an insufficient adaptation for survival and reproduction of the translocated individuals in their new environment. Therefore, for successful managing of populations of rare and endangered species, information regarding habitat requirements (Brussard 1991) is necessary, particularly for determining how habitat change influences species composition, and the identification of suitable sites for the establishment of conservation areas. Consequently, the best strategy for conservation of genetic diversity of endemics is preservation of the genetic integrity of gene resources by conserving them within the evolutionary dynamic ecosystems of the natural habitat. It assures not only the long-term survival under environmental conditions to which they are best adapted to, but also allows the maintenance of the adaptation and evolution processes of populations to their environments as well as the conservation of diversity at all levels, i.e. as ecosystem, species and genes. Especially, allowing co-evolution to occur will help to cope with future evolution in stresses in the crop.

Because most wild arabica coffee populations are utilized by individual farmers (Stellmacher 2007), sustainable management is difficult. Conservation of all populations is thus not possible, and decisions about which populations to preserve must be made. Therefore, information on the amount and pattern of genetic variation of forest coffee populations in Ethiopia is a crucial variable in the planning process of conservation. Furthermore, better information and better access to this information, will make it easier for coffee breeders to make use of wild relatives, which will add to their value and thus to the desirability of their conservation.

3 MATERIAL AND METHODS

3.1 Study area

The study was conducted in natural stands of *C. arabica* in patches of the Afromontane rainforest on the western and eastern highland plateaus of Ethiopia, which represent major residual areas of wild arabica coffee (Figure 2). Specifically, four distant habitats spanning the species natural range were chosen, namely Berhane-Kontir, Bonga and Yayu and Harenna Forest. While the latter is located on the eastern plateau of the highlands, all other sites can be found in the south-western part of the country, separated from Harenna by the Great Rift Valley.

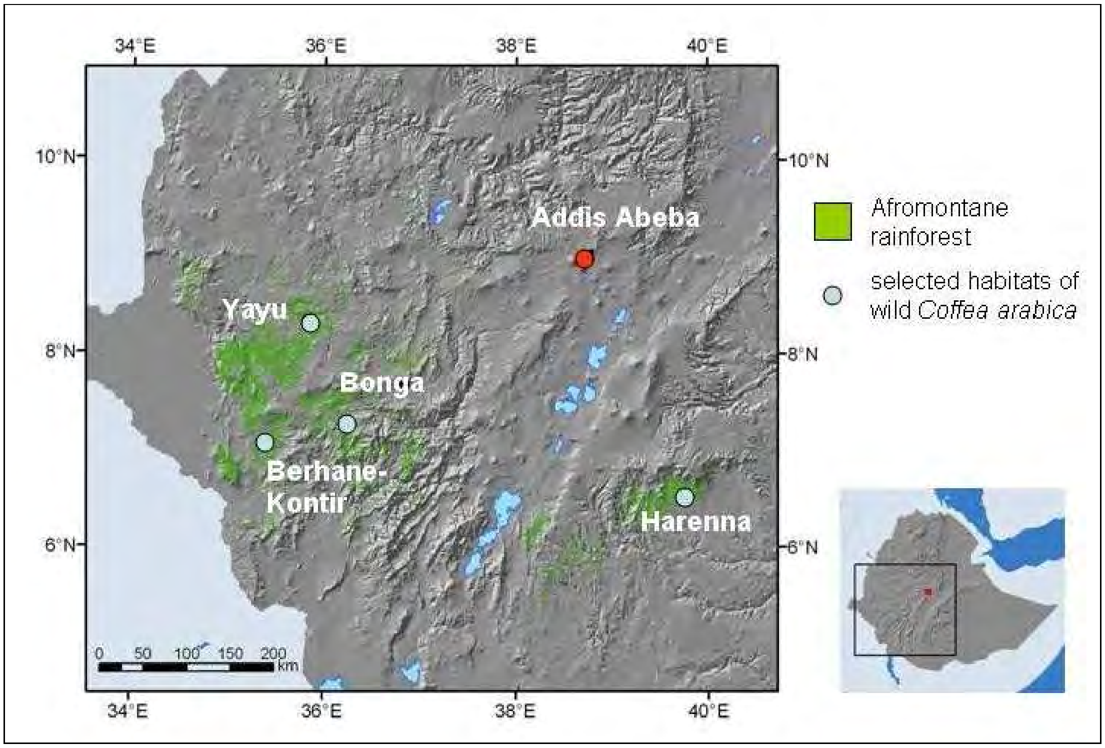

Figure 2: Location of the four selected wild *Coffea arabica* habitats in the Afromontane rainforest (left) and map of Ethiopia (right).

All study sites are classified as Afromontane rainforests (Friis 1992). Dystric Nitisols are the dominant soil association in this Afromontane rainforest region (FAO, ISCRIC et al. 1998). The natural stands were selected over a broad gradient of environmental conditions and located between altitudinal ranges of 1000 to 1800 m a.s.l.

Table 1: Location and environmental conditions of the four distant habitats of wild *C. arabica*.

habitat	Latitude *(N)*	Longitude *(E)*	Altitude *(m)*	Temperature* *(max)*	*(min)*	Station
Harenna	6°00'	40°00'	1420-1490	34	10	Dolo Mena
Bonga	7°13'	36°17'	1520-1780	26	12	Bonga
Yayu	8°22'	35°50'	1410-1470	28	13	Yayu
Berhane-Kontir	6°59'	35°36'	1040-1180	27	15	Mizan Teferi

*Source: Weather station next to the study sites (NMSA 2004).

The maximum air temperatures fluctuate between 26 and 34°C, while the minimum temperatures lie between 10 and 15°C. The distant habitats exhibit a high variability with respect to water availability (Figure 3; Table 2). A broad precipitation gradient extends from the relatively dry southern part, which receives 950 mm annual precipitation (Harenna) to the south-western regions with intermediate amounts (Bonga and Yayu), and annual precipitation as high as 2,100 mm in Berhane-Kontir.

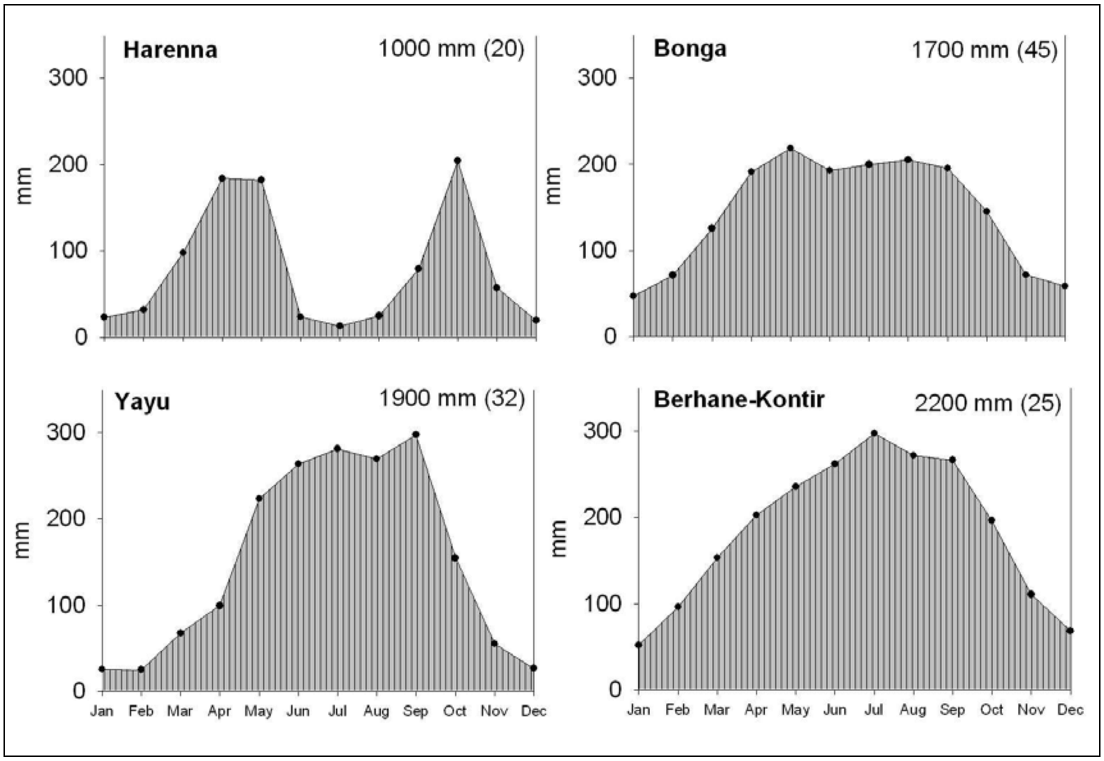

Figure 3: Long-term mean annual precipitation and distribution of the habitats of wild *C. arabica* in Ethiopia (values in parentheses indicate the length of the climatic record in years).

In all habitats, more than 85 % of the annual rainfall falls within the wet season; nevertheless, the geographic variability is accompanied by a high temporal variability with respect to precipitation and drought. Whereas the south-western habitats experience conditions of a mono-modal rainfall type, with a single dry and wet season per year, the Harenna Forest lies in an area with a bi-modal rainfall pattern showing a long rainy season in the beginning of the year and a prolonged phase of reduced water availability followed by a second wet period later in the growing season.

Table 2: Precipitation gradient spanning the habitats of wild *C. arabica* chosen for the study.

	annual precipitation		mean monthly precipitation		maximum drought length	
habitat	mean *(mm)*	CV *(%)*	dry season *(mm)*	wet season *(mm)*	mean *(days)*	CV *(%)*
Harenna	950 (20)	26	22	130	43	33
Bonga	1700 (44)	16	62	184	22	61
Yayu	1800 (32)	11	40	227	30	41
Berhane-Kontir	2100 (25)	13	72	222	18	41

*CV = (standard deviation x 100) mean (thus expressed as percentage)
Source: (NMSA 2004)

In addition, the sites vary in precipitation amount during the wet and dry season with the lowest dry season rainfall for sites with the lowest mean annual precipitation as found in Harenna. Also, the dry season length varies between the driest and the wettest site with longer maximum drought duration for the drier habitat (Harenna) and a shorter dry period for the habitat with highest annual precipitation (Berhane-Kontir). Furthermore, there is also a difference in the predictability of water availability that has to be taken into account, expressed as the coefficient of variation (CV). For annual precipitation, the CV represents the unpredictability of the rainfall amount received each year, whereas the CV of the longest annual drought represents a measure of the year-to-year unpredictability of drought duration. Both indices were greater for the drier site than for the wetter sites – a pattern typically seen in comparisons of spatio-temporal heterogeneity of water availability (Ehleringer 1994).

3.2 Experimental design

In order to evaluate the response of wild *C. arabica* to drought stress, two experiments were carried out by combining *in-situ* field measurements with an experimental approach under common-garden conditions *ex-situ*. The first experiment was designed to determine seasonal differences in the ecophysiological behavior of populations during a single dry and wet season in their natural habitat under varying weather conditions. In a second experiment, the plants examined under field conditions were used as seed collection sites for a drought-stress experiment where seedlings were grown under identical environmental conditions.

3.2.1 *In-situ* study: Ecophysiological response to seasonal changes in water availability under field conditions

Plant material

Within each of the four habitats along the environmental gradient, three field sites with natural vegetation and co-occurrence of *C. arabica* were identified (Table 3). These populations show natural germination in the understory of the Afromontane rainforest and were selected based on accessibility, altitude, age, stand characteristics and their degree of

Table 3: Stand characteristics of the wild *C. arabica* populations selected within the four habitats.

Habitat	Population	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	Aspect	Slope (%)
Harenna	1	6.483	39.755	1420	W	2-3
	2	6.487	39.753	1420	N	3
	3	6.498	39.745	1490	E	3
Bonga	1	7.313	36.056	1780	NW	4-6
	2	7.283	36.214	1520	S	3-6
	3	7.332	36.224	1660	NE	4-5
Yayu	1	8.399	35.795	1400	E	4-18
	2	8.398	35.796	1400	E	5-10
	3	8.400	35.795	1400	W	9-17
Berhane-Kontir	1	7.123	35.436	1040	NE	1-8
	2	7.120	35.438	1080	E	2-3
	3	7.071	35.425	1180	NE	5-7

human disturbance. In each population, 12 trees about 2 m in height and aged approximately 8 years were selected and permanently marked for repeated measurements over the seasons.

Treatments

Ecophysiological measurements were taken over a range of environmental conditions during the dry and wet seasons of a year to capture seasonal environmental variability of the populations. Therefore, two separate data sets were collected at each site. The first data collection was carried out in July and August 2003, which was characterized by normal wet season precipitation in all habitats, while the second field campaign in February/March 2004 reflected the dry season.

3.2.2 *Ex-situ* study: Ecophysiological response to drought stress under common-garden conditions

Plant material

The common-garden experiment was established at the experimental nursery site at the Ethiopian Agricultural Research Subcenter in Jimma (JARC), Ethiopia (7°36'N, 36°48'E at 1,750 m a.s.l.) in spring 2005. The site is located on former pasture land with a slope to the east with less than 4°. In March, mean daily minimum and maximum temperatures are 12°C and 28°C, respectively, whereas long-term annual precipitation is about 1600 mm (years recorded: 1986-2003).

From October to December 2003, ripe coffee cherries were obtained from the maternal plants of the populations used in the *in-situ* experiment. Seeds were kept in a conventional well-ventilated coffee seed storage room at JARC for about 15 weeks with average minimum and maximum temperatures of 10 and 20°C, respectively. On March 29 and 30, seeds were sown equidistantly at a uniform depth of 5 cm into individual 5.8 cm^3 pots filled with a homogeneous 3:1 mix of local topsoil (clay loam) collected from the nursery site and decomposed coffee husk compost. The pots were evenly spaced and randomly arrayed at the nursery site. They were kept under semi-controlled environmental conditions and uniformly managed under partial shade conditions. When necessary, irrigation was provided in order to ensure the maintenance of field capacity, supplementary to precipitation. In addition, weeding and insects were monitored and controlled. Emergence began 55 days after sowing and most seedlings had one true leaf after another 112 days. To provide a covariate to account for initial seedling size differences, the leaf area and plant height was measured at the time before treatment start. This metric also accounts for any stress effects before transplanting.

Treatments

Four different treatments were applied in the experiment. Two light intensity regimes (shade and open sun) as well as two soil moisture treatments (irrigated and non-irrigated) were imposed. For the shade treatment, shade nets were erected horizontally above the seedlings while unshaded plants were grown under natural open sun conditions. Water was withheld from half of the pots in order to simulate a fast soil drying period, whereas the well watered plants were irrigated regularly in 4-day-intervals to ensure moisture conditions corresponding to soil water availability in the field during wet periods.

The four different growth conditions were defined as follows: (i) well watered, shade; (ii) well watered, open sun; (iii) drought-stressed, shade; (iv) drought-stressed, open sun (Table 4).

Table 4: Treatments in the *ex-situ* experiment

		Soil moisture availability	
		irrigated	non-irrigated
Light intensity	low light	well watered, shade,	drought-stressed, shade
	high light	well watered, open sun	drought-stressed, open sun

Fifty-five days after emergence, 80 seedlings of uniform height were chosen from each population and randomly assigned to the four different treatments, with every population represented by 25 replicates within each treatment. The experimental set-up thus consisted of a total of 1200 plants (4 habitats x 3 populations x four treatments x 25 replicates).

A split-split-plot design was applied with the following components: light availability represented the main plot, while soil moisture availability was a sub-plot within the irradiance regime, and population was a split-plot within the four growth regimes produced by the combination of light availability and moisture availability. The treatments were imposed over a 17-day period.

3.3 Measurements

3.3.1 Environmental parameters

Atmospheric and soil moisture conditions

Weather data were collected during the 2003-2005 growing seasons using automated weather stations. Four climatic monitoring stations were established at all four habitats in an open area to avoid interference from trees. Climatic differences between habitats were quantified by measuring the air temperature, relative humidity, precipitation, wind speed and global radiation (SP LITE Silocon Pyranometer, Kipp & Zonen, Delft, The Netherlands). Measurements were taken every minute, averaged and stored every 15 min with a data logger (Mikromec multisens, Technetics, Freiburg, Germany).

Additionally, volumetric soil moisture was measured using a soil moisture sensor (ThetaProbe^{TM}, Sensor Type ML1, Delta-T Devices, Eijkelkamp Agrisearch Equipment, The Netherlands). For comparison of the wet and dry seasons in the *in-situ* study, measurements were made with 12 replications per population within each habitat per season, while in the common-garden experiment; records were taken every four days per treatment with six replications per habitat.

Leaf area index (LAI)

Light transmittance through the plant canopy of the upper canopy layer in the forests to the plants in the shrub layer was measured using a LAI-2000 canopy analyzer (Li-Cor inc., Lincoln, NE). Photosynthetic active radiation was measured 1.2 m above ground level at five randomly spaced points along each of the three populations per habitat.

Foliar and soil nutrient analysis

Fully expanded leaves from the uppermost part of the crowns of the selected experimental coffee trees per population were sampled during the wet season in 2003. Foliage samples were mixed to make a single sample from each population and dried at 70°C to constant weight and ground to pass a 1-mm sieve. They were then analyzed for total concentration of N by a micro-Kjeldahl procedure and for P, Ca, Mg, K, S, Fe, Mn, Mol, Cu, Zn and Na by inductively coupled plasma emission spectroscopy.

Furthermore, soil samples of the rooting zone were collected from each plot. The soils were then analyzed for organic C by chromatic acid digestion and spectrophometric methods, whereas total N was determined by micro-Kjedahl, K by Technicon continuous-flow flame emission spectrophotometry, and P, Ca and Mg by inductively coupled plasma emission spectrometry.

3.3.2 Gas exchange parameters

Gas exchange measurements were obtained using a portable infrared gas analyzer (LCpro, ADC Bioscientific Ltd., Hoddesdon, UK) with flow rate (200 cm^3 min^{-1}), pressure (100 kPa), and leaf area (6.30 cm^2) set the same for all measurements. Net photosynthetic rate (A_{net}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}) and transpiration (E, mmol m^{-2} s^{-1}) were measured. Instantaneous water-use efficiency (WUE_i) was calculated as the ratio of net assimilation (A_{net}) to transpiration (E). For both experiments, two different types of leaf gas exchange measurements were carried out, i.e., diurnal and seasonal variation of gas exchange activity and light response curves of photosynthesis. During the wet season in the *in-situ* study, frequent heavy rainfall over the day made measurements of diurnal change in gas exchange parameters impossible. Therefore, the comparison of dry and wet season values is based only on midday measurements of porometry. Diurnal variation of gas exchange parameters were conducted in the *ex-situ* study as well as on field-grown plants during the dry season at 2-h intervals from 8:00 to 18:00 h over 2 consecutive days per population. All measurements were made on 5 individuals per population during the dry and the wet season (*in-situ*) and on 2 plants per population per treatment (*ex-situ*), respectively. Two new fully expanded leaves were excised from each plant and measured by placing the mid-portion of the fascicles in the cuvette, while values were allowed to stabilize before measurements were taken. Measurements were performed on the same leaves as those used for chlorophyll fluorescence measurements.

Photosynthetic light response curves were measured on leaves from 2 seedlings per treatment in the *ex-situ* study with an attached red LED light source (ADC). Measurements began with approximately 5 minutes of saturating light (1500 µmol m^{-2} s^{-1}) followed by 9 incremental reductions until the irradiance reached 10 µmol m^{-2} s^{-1}. Use of decreasing light rather than increasing light reduces the equilibrium time required for stomatal opening and photosynthetic induction (Kubiske and Pregitzer 1996). Preliminary trials indicated that photosynthetic rates reached steady state within 2 min following each incremental decrease in light. The measurements were restricted to the hours between 10 and 15 h on sunny days to minimize diurnal effects on photosynthesis.

In addition to the ecophysiological measurements, micro-environmental parameters at the natural stands of the selected *C. arabica* populations were recorded. Light availability was measured as photosynthetically photon flux density (PPFD, µmol m^{-2} s^{-1}), reflecting ambient conditions at the specific date of measurement. Air temperature in the chamber was maintained within 1 to 2°C of ambient by varying the speed of the chamber fan. Leaf-to-air vapor pressure difference (VPD; kPa) was computed as:

$$\mathrm{VPD} = [e_{sat}\,(T_{air}) - e_{air}]\;/\;\mathrm{pressure\ (mb),\ where}$$
$$e_{sat} = 6.1078 \cdot \exp\,[(17.269 \cdot T_{air})\;/\;237.3 + T_{air}],\ \mathrm{and}$$
$$e_{air} = e_{sat} \cdot (\mathrm{RH}\;/\;100)$$

following (Campbell and Norman 1998). In addition, air entering the system passed through a 4-l buffer volume, and was scrubbed of CO_2 by passing it through a column of soda lime. The CO_2 concentration was then adjusted to 350-360 µmol m^2 s^{-1} by the system's CO_2 injector.

3.3.3 Chlorophyll α fluorescence measurements

In the *ex-situ* study, chlorophyll *a* fluorescence measurements were performed with a pulse-amplitude modulated fluorometer equipped with a leaf clip holder (Mini-PAM, Heinz Waltz, Effeltrich, Germany) on the same plants as those used for leaf gas exchange measurements. Measuring light and a saturated light pulse with a PPFD of 6000 µmol m^{-2} s^{-1} were applied through a fiberoptic cable, and fluorescence emitted from the leaf was measured with the same device. In order to evaluate the role of dynamic and chronic phases in the inactivation process of photosystem II (PS II), two different measurements were done: Induction curves (IC) and rapid light response curves (RLC).

Measurements of potential quantum yield via induction curves (IC) were done on two plants per population for each sampling time and treatment. The leaves were pre-conditioned in the dark for 30 min and illuminated for 5 seconds to induce fluorescence. Minimum (F) and maximum (F_m) chlorophyll a fluorescence levels were measured and maximum quantum efficiency of PS II photochemistry ($F_v/F_m = (F_m - F_o)/F_m$) was determined (Bilger, Schreiber et al. 1995; Rascher, Liebig et al. 2000). The effective quantum yield of PS II was calculated as $(F_m'\text{-}F)/F_m' = \Delta F/F_m'$, where F is the fluorescence yield of the light-adapted sample and F_m' is the maximum light-adapted fluorescence yield when a saturating light pulse was superimposed on the prevailing environmental light levels (Genty, Briantais et al. 1989).

Additional parameters of photosynthetic activity in light-adapted leaves were obtained from rapid light-response curves (RLC). Each sampling time, population and treatment were represented by 4 replicates. Electron transport rate (ETR) was determined according to the following formula: $\mathrm{ETR} = \varphi P \times 0.84 \times 0.5 \times \mathrm{PAR}$, where $\varphi P = (F_m' - F_t)/F_m'$ is the effective PSII quantum yield, 0.84 is the assumed absorption coefficient, 0.5 is the correction for PS II absorbing photons, and PAR is the photosynthetic active radiation. During the RLC measurement, the samples were exposed to nine incremental steps of irradiance ranging from 0 to 600 µmol photons m^{-2} s^{-1} with a saturation pulse of 10 s of irradiance at each incremental step. RLCs were fitted using an exponential function in order to obtain the initial slope of $\Delta F/F_{m'}$ and ETR, maximum electron transport rate (ETR_{max}) and photosynthetically active radiation at saturation of photosynthesis ($PPFD_{sat}$) according to (Platt, Gallegos et al. 1980).

$$P = Ps\,[1 - \exp(-\alpha\, Ed / P_S)]\, \exp(-\beta\, E_d / P_S)$$

E_d is the down-welling irradiance (wavelength 400-700 nm), P_S is a scaling parameter defined as the maximum potential ETR in the absence of photoinhibition, α is the initial slope of the light curve before the onset of saturation providing a measure of the efficiency of light utilization, and β is the slope of the light curve beyond the onset of photoinhibition. In the absence of photoinhibition (β=0) equation 1 simplifies to:

$$P = Ps\,[1 - \exp(-\alpha\, Ed / P_S)]$$

The maximal rate of relative ETR at light saturation is termed the photosynthetic capacity, Pm, and can be derived as

$$P_m = P_S\,[\alpha / (\alpha + \beta)]\,[\beta / (\alpha + \beta)]\,\beta/\alpha$$

3.3.4 Leaf water relations

All leaf water relation parameters were repeatedly measured on individual mature trees without compromising the health of the plant throughout the season in the *in-situ* study. In the common-garden study, however, seedlings were too small to be measured repeatedly on identical individuals without causing damage, because leaf removal would have damaged the seedlings. Therefore, measurements in the *ex-situ* study were done on different individuals within the specific population and treatment for each sampling date.

Leaf water potential (ψ)

Leaf water potential (ψ) was estimated using a pressure chamber (SoilMoisture Equipment Corp., Santa Barbara, CA, USA) as described by Scholander (1965). On each sampling date, ψ measurements were taken from the terminal twigs of selected plants and monitored at midday (ψ_{md}; 12:00 to 13:00 h, local time) and the following predawn (ψ_{pd}; 05:00 h) on the uppermost fully expanded leaves. Measurements were made on 8 trees per population with 3 leaves per tree (*in-situ* study) and on 2 seedlings per population (*ex-situ* study), respectively. The short petioles of coffee species did not protrude beyond the rubber seal of the pressure bomb. Therefore, in order to standardize the measurements, shoots bearing 2 leaves were used in all species, instead of single leaves. Measurements were performed within 10–25 seconds after collecting the samples. Pressure was applied to the chamber at a rate of 0.05 MPa s^{-1} (Turner 1981).

Leaf osmotic potential (π)

The samples for measurements of osmotic potential were taken from the same branches as the samples for the measuring water potential. The leaves were killed in order to avoid enzymatic changes in the cells (Mitlöhner 1998). Sample leaves were then immediately sealed in plastic bags, placed in an insulated container and transported to the laboratory. Dried leaf samples were ground to a fine powder in a ball mill (Retsch, Vienna, Austria) and extracted with hot water (55°C for 12 h) and processed following the method of (Mitlöhner 1998). The sap was removed by centrifugation (40,000 g for 15 min; Beckman AvantiTM 30, Klosterneuburg, Austria) and collected in tubes that were immediately sealed and stored on ice. Leaf osmotic potential of the extracted sap was determined by freezing-point depression with a freezing-point osmometer (Osmomat 030, Gonotec, Berlin, Germany) according to (Kreeb, Richter et al. 1989) with a measuring error of ± 0.2 MPa.

Relative water content was determined as described by (Barrs and Weatherley 1962) and the turgor potential (*P*) was calculated from the linear relationship between osmotic and pressure potential changes in response to water potential ($P = \psi - \pi$).

3.3.5 Carbon isotope discrimination ($\delta^{13}C$)

Foliar carbon isotope discrimination ($\delta^{13}C$) was determined on leaves collected at the end of each field campaign in the *in-situ* study, thus comprising dry and wet season estimates of variation in water-use efficiency of the coffee plants, as well as at the end of the drought-stress experiment in the *ex-situ* study. Specifically, 20 plants were selected at each population, and 4 leaves per tree collected from all cardinal directions were bulked into a single sample per plant. At the end of the *in-situ* study, wood material was collected for $\delta^{13}C$ analysis by selecting cross-sections of stems from six trees per population that were also used for the other ecophysiological measurements.

Samples were oven dried at 70°C for 72 h and the dried pooled samples were ground into a fine powder in a matrix mill (MM 301, Retsch, Haan, Germany). Analyses of $^{13}C/^{12}C$ were carried out with a mass spectrometer (Finnigan Delta-S) at the Institute of Plant Nutrition, University of Bonn, Germany. The carbon isotope ratios were expressed as $\delta^{13}C$ (‰) against the Chicago Pee Dee Belemnite (PDB) standard (Farquhar, Ehleringer et al. 1989) computed as $\delta^{13}C = [(R_{sample}/R_{standard}) - 1]\ 1000)$, and $R = {}^{13}C/{}^{12}C$ (Craig 1957). Reproducibility was 0.1 with well-homogenized cellulose standards.

3.4 Statistical analysis

The statistical analysis of the data was performed by analysis of variance (ANOVA) using the SPSS General Linear Model procedure (SPSS version 12, SPSS Inc., Chicago, IL, USA). Tests for normality and homogeneity of variance indicated that no special transformations of the data were needed (Sokal and Rohlf 1995). Test of equality of averages using Bonferroni (t-test) was applied equally (the same letters indicate that averages are not significantly different, and the alphabetic order corresponds to decreasing values). Significance was as follows: ns, not significant; *, significant ($P < 0.05$); **, highly significant ($P < 0.01$); ***, extremely significant ($P < 0.001$).

In-situ **experiment**

The ecophysiological behavior of the populations measured *in-situ* was analyzed by the GLM Univariate ANOVA procedure and the effect of season, habitat, population (nested within habitat) and the interactions among these were evaluated. When appropriate, the dependency of ecophysiological parameters on environmental factors was evaluated with regression analysis.

Ex-situ experiment

A nested mixed-model ANOVA was used to estimate the significance of the following factors: (1) habitat, testing for differentiation in character means among habitats independent of the environment; (2) population (nested within habitat), testing for genetic differentiation in character means among accessions independent of the environment (3) treatment, estimating overall phenotypic plasticity independent of genetic effects; (4) population*treatment interaction, testing for the existence of genetic differentiation for plasticity among populations. Treatment and habitat were considered as a fixed effect, while population was treated as a random effect.

For all traits with a significant treatment, habitat*treatment or population (nested within habitat)*treatment term, reaction norms were plotted, showing the phenotypic state at each point along an environmental gradient (Gupta and Lewontin 1982). The phenotypic state was represented by the regression line of the plot of trait expression against environment. The magnitude of phenotypic plasticity is evaluated as the slope of reaction norm of the trait (Gianoli 2001).

Furthermore, the *ex-situ* measurements were also used to calculate a 'drought resistance index' (*S*) for each measured parameter using the following relationships:

$$S = (1 - Y_S/Y_P) / (1 - X_S/X_P) \quad \text{(Fisher and Maurer 1987)}$$

where Y_S is the mean performance of a certain population (computed over 6 plants) under drought stress, Y_P the mean performance of the population under irrigated conditions, and X_S and X_P are the mean performances of all populations under these specific treatments.

Phenotypic correlations among physiological traits were estimated as the Pearson product-moment correlation (r) using the subset of the most contrasting populations within the specific testing environment. The treatment-specific correlation matrices using the full dataset of all plants were also used for principal components analysis (PCA) in order to visualize the sets of co-varying traits in each environment and to determine the extent of differentiation of the multivariate phenotype of populations.

4 RESULTS

4.1 Habitat conditions of wild *Coffea arabica* populations

4.1.1 Climatic conditions

Environmental conditions during the study period showed that distant habitats of wild *C. arabica* populations differed considerably in their range of climatic conditions following the overall patterns of long-term averages (Table 5). Mean annual precipitation was the main environmental variable that differed among habitats, which varied from a low of 800 mm year^{-1} at Harenna to a high of 2100 mm year^{-1} at Berhane-Kontir following the patterns of long-term averages, whereas Bonga and Yayu showed intermediate rainfall amounts. Additionally, habitats differed in seasonality of water availability with clear differences between bi-modal and mono-modal rainfall types. Habitats with two distinct wet seasons as found in Harenna corresponded to two prolonged periods with reduced precipitation amounts whereas the other habitats were characterized by a single dry and wet season within a year (Table 5, Figure 4). Monthly precipitation during dry seasonal ranged from 21 to 75 mm year-1 and showed to be inversely related to maximum drought length in Harenna, Bonga and Yayu with sites with lowest dry season precipitation exposed to longer dry periods. Surprisingly, the habitat with the highest annual rainfall pattern, Berhane-Kontir, exhibits the longest dry period experienced by the plants. However, it exceeded the long-term records in dry season length by 277 %, which correspond to abnormal severe drought conditions during the study period.

Table 5: Annual and seasonal precipitation characteristics of the four native habitats of wild *C. arabica* populations during the field campaign of the *in-situ* study (2003 - 2005)

		Harenna	**Bonga**	**Yayu**	**B.-Kontir**
annual precipitation	*mm*	800	1600	2000	2100
+/- long-term	*%*	81	93	112	95
rainfall type		bi-modal	mono-modal	mono-modal	mono-modal
monthly dry season precipitation	*mm*	21	50	35	75
+/- long-term	*%*	90	80	85	105
maximum drought length	*days*	45	21	35	68
+/- long-term	*%*	104	95	116	277

4.1.2 Leaf nutrient analysis

Leaf nutrient contents from wild *C. arabica* plants grown in-situ that are represented in Table 6 showed a considerable variation among distant habitats. Excessive levels of nutrient contents could not be revealed, generally plants showed to exhibit adequate quantities of nutrients. In same cases however, leaf analytical data identified macro- and micronutrient deficiencies. Critical values in potassium were found for Harenna whereas Berhane-Kontir and Yayu showed high deficiency in calcium and magnesia content. Furthermore, all study sites revealed critical levels of mangan, which showed to be reduced by almost 50% compared to level that are known to be adequate for optimal growing conditions in coffee production.

Table 6: Leaf nutrient content in four distant habitats of wild *C. arabica* obtained in 2004

	Harenna		**Bonga**		**Yayu**		**B.-Kontir**		***adequate****
P (%)	0.07	c	0.12	b	0.11	c	0.15	a	*0.10-0.20*
K (%)	1.80	c	2.2	b	2.1	b	2.7	a	*1.90-2.70*
Ca (%)	1.60	a	1.2	b	0.33	c	0.02	c	*1.00-1.40*
Mg (%)	0.35	a	0.33	b	0.26	bc	0.28	ac	*0.31-0.36*
Na (%)	0.03	bc	0.02	a	0.02	a	0.03	ab	
Zn (ppm)	13.9	a	11.5	b	9.7	c	11.1	b	*8.00-16.00*
Mn (ppm)	61.0	b	67	b	59	b	98	a	*120-210*

*according to (Malavolta 1990)

4.1.3 Soil nutrient analysis

Analysis of physical and chemical soil properties revealed that there are habitat-to-habitat differences in pH and level of nutrients (Table 7). The soils of the distant habitats are acidic to slightly acidic with a pH range of 4.7 and 6.8. Soil organic matter varied between 2.18 % in Berhane-Kontir and 4.90 % in Harenna, respectively. Furthermore, soils in forests of Harenna and Bonga are characterized as rich in basic exchangeable cations with limited available phosphorus, whereas Berhane-Kontir and Yayu showed low level of available potassium. Furthermore, habitats differed in their physical properties. Patterns of soil texture revealed Harenna being rich in clay in contrast to the other sites while in Berhane-Kontir forest a high content of sand was found. Higher silt content found in Bonga and Berhane-Kontir in contrast to Harenna and Yayu are in line with their higher degree of field capacity ranging from 33.7 to 42.5.

Table 7: Chemical and physical soil properties and canopy structure features at the four habitat of wild *C. arabica* populations obtained in 2003.

	Harenna	**Bonga**	**Yayu**	**Berhane-Kontir**
pH (H_2O)	*6.8*	*4.7*	*5.0*	*5.9*
Organic matter (%)	4.90	3.70	2.60	2.18
Available N (mg kg^{-1})	*0.37*	*0.18*	*0.12*	*0.20*
Available P (mg kg^{-1})	*0.90*	*0.35*	*12.84*	*4.65*
Available K (mg kg^{-1})	*1.1*	*1.3*	*0.4*	*0.3*
CEC (meq $100g^{-1}$)[a]	*29.2*	*30.2*	*26.0*	*20.3*
Texture (%) S-Si-Cl	*22:15:63*	*24:22:54*	*39:16:45*	*15:30:55*
Field capacity (%)	*38.0*	*42.5*	*33.7*	*39.3*
overstory LAI (canopy trees)	1.76	2.21	2.14	1.24
coffee management	semi-forest coffee	forest coffee	forest coffee	semi-forest coffee

[a] CEC = cation exchange capacity

Leaf area index (LAI) as a measure of canopy light penetration was used as an indirect estimate of the overstory leaf area relative to ground area. A high LAI indicates low light levels penetrating the canopy, while a low LAI is a sign for high light penetration through the canopy of the overshading trees. Results showed that sites differed in their degree of LAI variation and that high canopy light penetration correlates with the management intensity of the coffee production system with intensive land management resulting in a more open overstory canopy.

4.2 *In-situ* study: Ecophysiological response to natural drought conditions in field-grown wild *Coffea arabica* populations

4.2.1 Environmental conditions

Specific climatic conditions prevailed during the two field campaigns of the *in-situ* study are illustrated in Figure 4. Whereas temperature fluctuated within a fairly constant range during the year with only slight increases during periods of reduced water availability, monthly precipitation amounts showed a clear seasonal distribution, and as indicated, dates selected for *in-situ* measurements captured this contrasting precipitation pattern of dry and wet season.

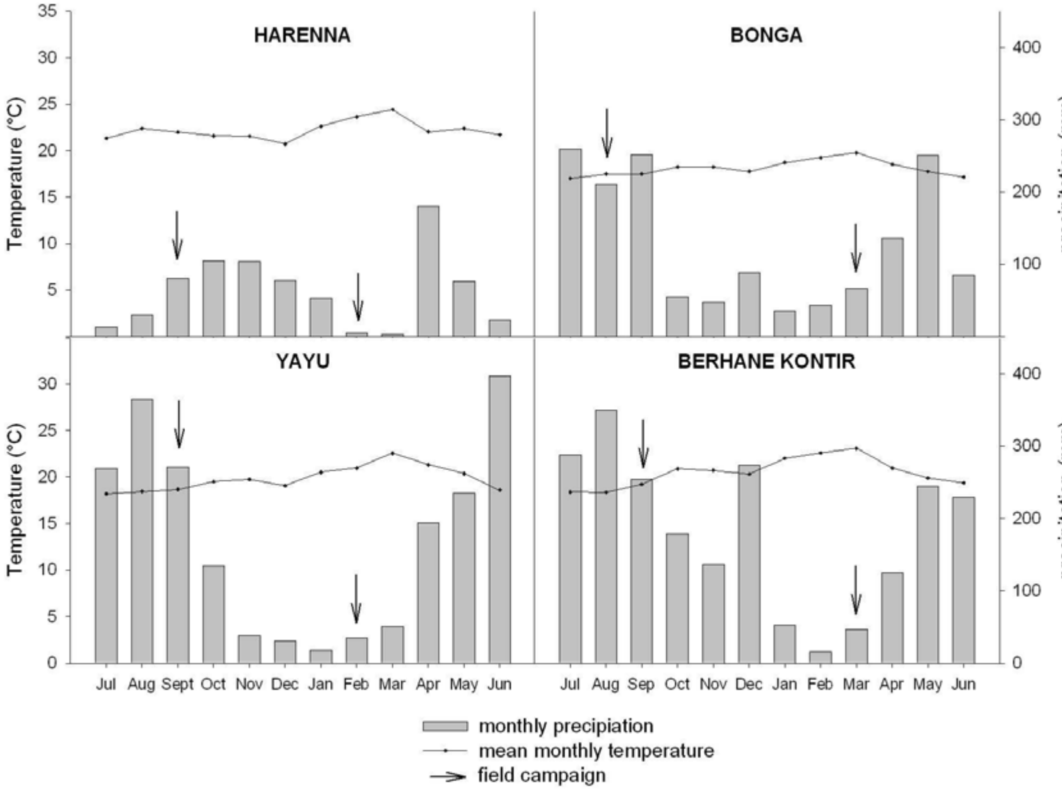

Figure 4: Seasonal variation in precipitation and temperature patterns at the four habitats of wild *C. arabica* populations during the study year of the *in-situ* study (July 03 to June 04)

Also, other micro-climatological variables revealed that measurements of the two field-campaigns of the *in-situ* study covered seasonal extremes in environmental conditions (Figure 5), therefore being valid to quantify seasonality in plants physiological behavior. General trends across seasons were prominent for soil moisture conditions (SMC), photosynthetic photon flux density (PPFD) as a measure of light intensity, air temperature (T_{air}) and leaf-to-air vapor pressure deficit (VPD). Soil water content decreased ($P < 0.05$) at all south-western habitats from a high of around 49 % during wet season to less than 16 % in the period of reduced moisture availability (Figure 5 a). Temporal variation in soil moisture availability was also valid for the Harenna habitat ranging from around 16 % to more than 21 %, however, differences were less profound and relatively low in comparison to the other habitats. Whereas seasonality had no effect on variation of light intensity at midday (PPFD; Figure 5 b) in Bonga forest, PPFD was lowered by approximately 50 % in the wet season in contrast to the period of reduced moisture availability at the other habitats. The period of higher light intensity coincided with elevated levels of midday air temperature (T_{air}) ranging from about 31 to 39°C and exceeding values during the wet period, which varied from 27 to 33°C (Figure 5 c).

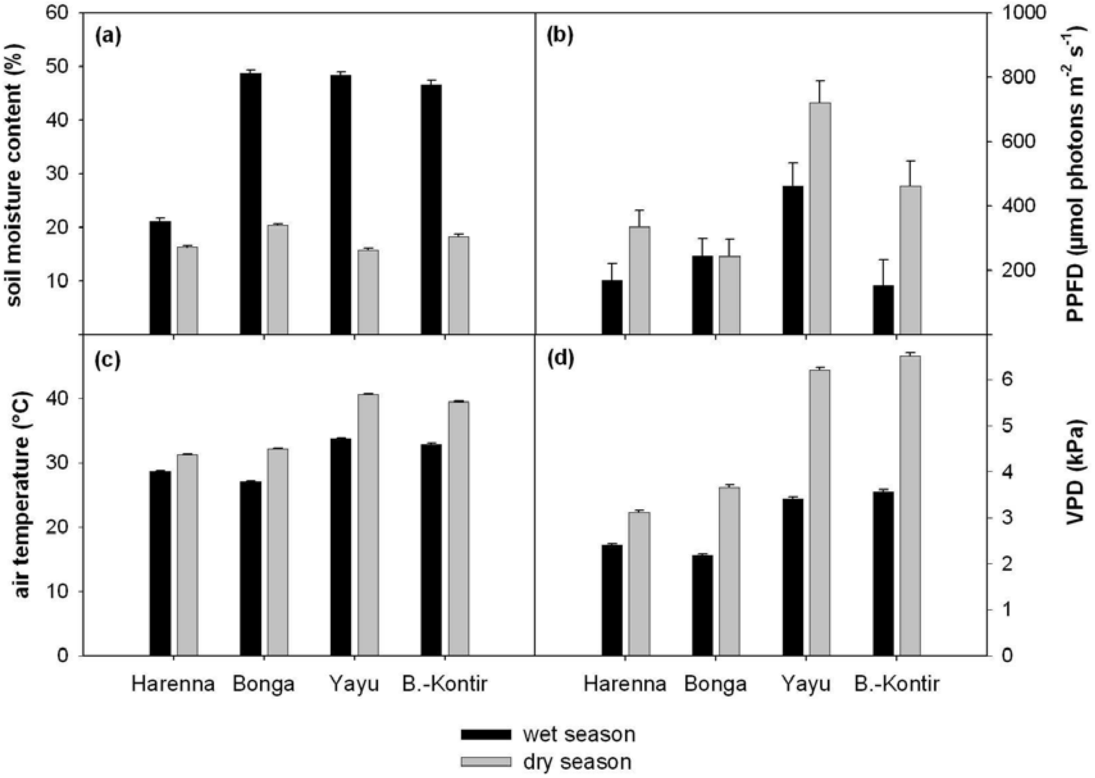

Figure 5: Environmental conditions of (a) soil water content (%), (b) photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}), (c) air temperature (°C) and (d) leaf-to-air vapor-pressure deficit (VPD, kPa) at four wild *C. arabica* habitats measured at midday during a single wet and dry season in 2003/2004 (microclimatic parameters were derived from gas exchange measurements). The values represent means ± S.D.

Specifically, a significant habitat by season effect (data not shown) revealed differences among the selected forest sites with higher air temperature for Yayu and Berhane-Kontir in contrast to the other sites in both seasons sampled. The leaf-to-air vapor pressure deficit (VPD; Figure 5 d) followed a similar pattern like the temperature differences with high maximum values during dry season ranging from 3.1 to 6.5 kPa in contrast to 2.2 – 3.6 kPa as revealed during wet season.

Besides the variation of environmental conditions over seasons, there was also a clear pattern of diurnal changes in atmospheric conditions in the dry season (Figure 6). Alteration in light intensity measured as photosynthetic photon flux density (PPFD) over a day started with early morning values of around 150 µmol m^{-2} s^{-1} followed by a maximum around midday ranging from 240 to 730 µmol m^{-2} s^{-1} and decreased again to 70 µmol m^{-2} s^{-1} in the afternoon. However, no habitat specific differences were revealed and the occurrence of understory sunflecks in the lower canopy of the forests resulted in relatively high values of standard deviation.

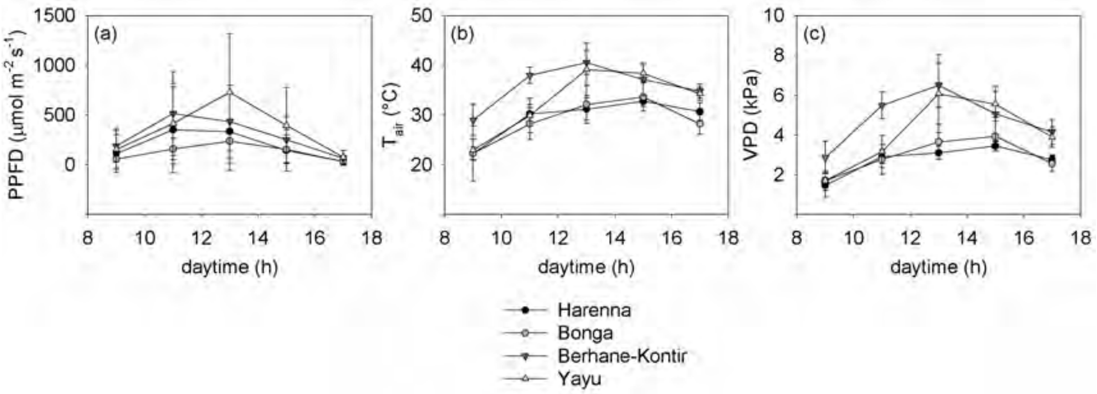

Figure 6: Diurnal patterns of (a) photosynthetic photon flux density (PPFD, mol m^{-2} s^{-1}), (b) air temperature (T_{air}, °C) and (c) leaf-to-air vapor pressure deficit (VPD, kPa) of the four habitats of wild *Coffea arabica* during dry season in 2004.

With respect to diurnal variation of air temperature (T_{air}, Figure 6 b), morning values differed among the study sites exhibiting highest values for Berhane-Kontir with 29°C whereas the other sites showed rather moderate values of 24°C. Around noon, all habitats increased to maximum values of 32° and 42°C, respectively, followed by 28° to 35°C in the afternoon. The diurnal pattern of leaf-to-air vapor pressure deficit (VPD) almost mirrored the varying course of air temperature ranging from minimum values of less than 2 kPa at all sites to a midday peak of 4 kPa in Harenna and Bonga and to 6 kPa in Berhane-Kontir and Yayu, respectively (Figure 6 c).

4.2.2 Leaf water relations

Seasonal patterns of predawn and midday leaf water potential (Ψ) of the four distant habitats are shown in Figure 7. At midday (Figure 7 a), plants showed to differentiate among seasons as well due to habitat conditions. Dry season values of midday Ψ were consistently lower than during wet season indicating that plants were strongly affected by drought stress during midday in periods characterized by reduced moisture availability and lowered atmospheric water contents. Moreover, midday leaf water potential measurements revealed that populations differed in their degree of seasonal variation. Whereas the midday values in the wet season were in the same range for all south-western habitats such as Bonga, Yayu and Berhane-Kontir a relatively low record of -1.2 MPa in Harenna populations expressed that plants experienced a certain degree of drought stress at this time. In dry season however, midday leaf water potential were in a similar range for coffee populations of all habitats studied reaching minimum values of almost -2.0 MPa. The closely related low values found in Harenna however showed that the plants were exposed to a certain degree of drought stress during both seasons sampled, while the other sites show a clear contrasting seasonal pattern in their leaf water potential.

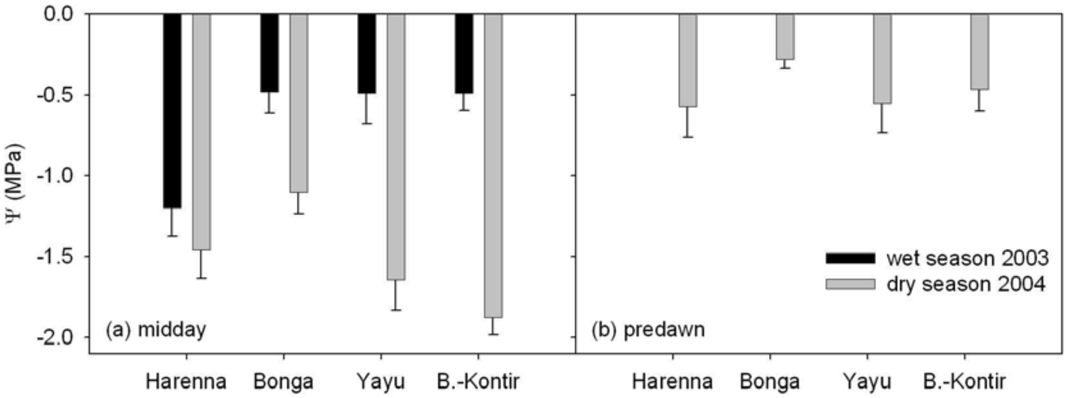

Figure 7: Seasonal changes in (a) midday and (b) predawn leaf water potential parameters (Ψ) of the wild *C. arabica* populations from different habitats during dry and wet season in 2003/2004.

While logistically constraints during field campaign in the wet season made the recording of predawn data of leaf water potential impossible at this time, results of the measurement during the period of reduced soil moisture availability revealed relatively high Ψ for all populations irrespective of their origin (Figure 7 b). Specifically, levels of predawn measurements during dry season field campaign were similar to the predawn Ψ values recorded in wet season; therefore no signs of serious drought stress affection of the plants were obvious.

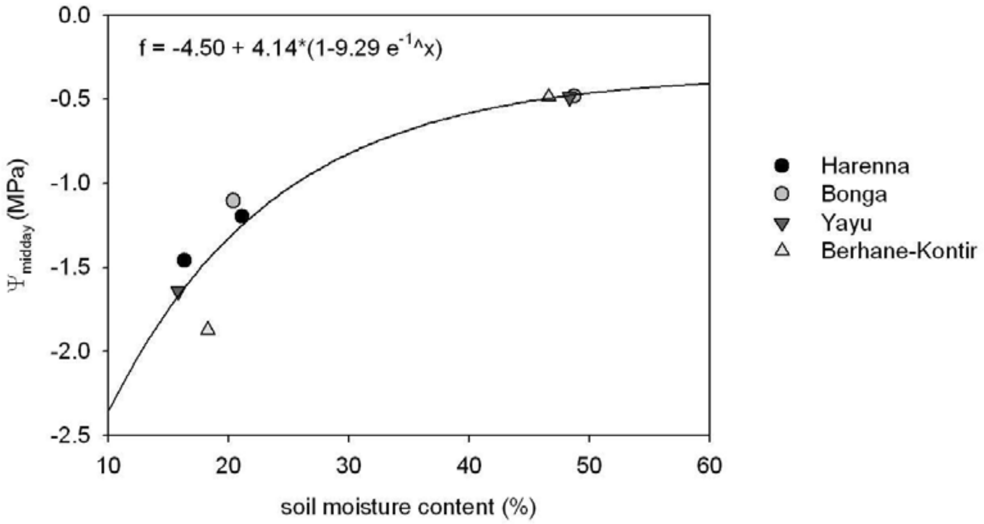

Figure 8: ***In-situ*** **midday leaf water potential (Ψ_{md}) of wild *C. arabica* populations from different habitats in response to soil moisture content (%) over season (dry and wet season)**

Furthermore, plants' specific behavior with respect to leaf water potential (Ψ) changes throughout the day during the period of reduced soil moisture availability can be seen in Figure 8 a, specified as the relationship between midday and predawn Ψ. In contrast to plants from Berhane-Kontir, Bonga, Harenna and Yayu provenances exhibited closely

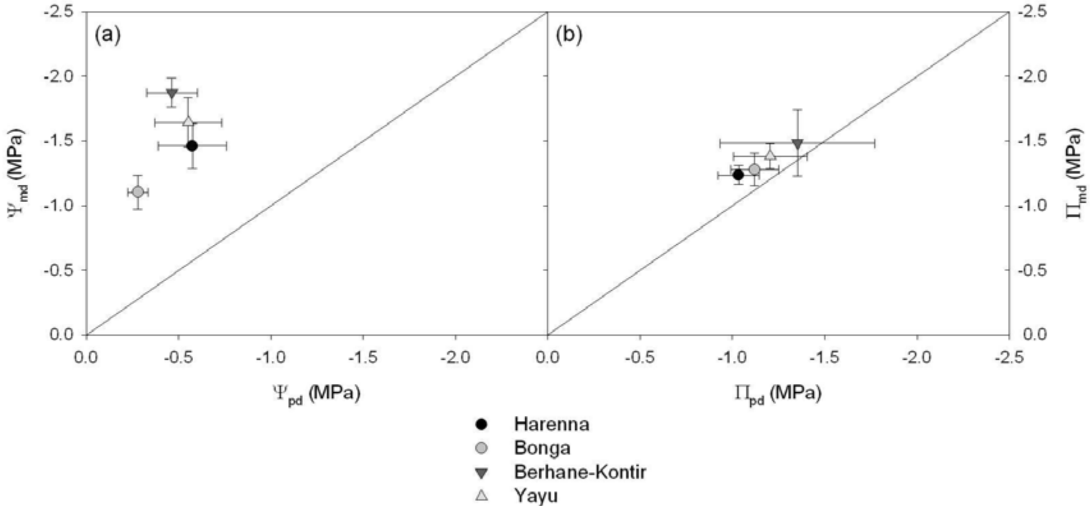

Figure 9: **Relationship between midday (md) and predawn (pd) measurements of (a) leaf water potential (Ψ) and (b) leaf osmotic potential (π) of wild *C. arabica* populations from different habitats during the dry season in 2004**

related midday and predawn values with a narrow range revealed by close distance to the bisecting line. The Berhane-Kontir plants however, controlled the extent of their daily variation in contrast to the other habitats and maintained a quite high range during dry season, hence showing the capability to recover from aggravated midday leaf water status over night. With regard to diurnal change in leaf osmotic potential (Figure 9 b), there were no great fluctuations in osmotic potential between midday and predawn measurements for all populations.

4.2.3 Gas exchange measurements

Leaf gas exchange characteristics differed over seasons as shown by a significant influence of time effect ($P<0.001$; Table 8). Generally, net photosynthetic rate (A_{net}), transpiration (E) and stomatal conductance (g_s) measured during period of sufficient soil moisture supply were higher at all study sites compared to the dry season (Figure 10). Average wet season A_{net} at midday ranged from 3.3 to 4.6 µmol m^{-2} s^{-1} while there was a reduction to 1.07 to 3.05 µmol m^{-2} s^{-1} during the dry period. Transpiration rates also decreased in dry season, however reduction due to reduced soil moisture availability was more pronounced than in A_{net}, resulting in greater instantaneous water-use efficiency (WUE_i) during dry season.

Table 8: Repeated measures ANOVA for *in-situ* net photosynthetic rate, transpiration rate, stomatal conductance and instantaneous water-use efficiency measured at midday in wild *C. arabica* populations from different habitats during dry and wet season.

Source		df	Type III	F	*P*	
Net photosynthetic rate (A_{net})						
	season	1	293.97	147.43	0.00	***
	season*habitat	3	25.92	13.00	0.00	***
Transpiration rate (E)						
	season	1	184.56	711.76	0.00	***
	season*habitat	3	5.19	20.02	0.00	***
Stomatal conductance (g_s)						
	season	1	0.60	797.91	0.00	***
	season*habitat	3	0.01	10.22	0.00	***
Water-use efficiency (WUE_i)						
	season	1	20.66	39.80	0.00	***
	season*habitat	3	6.91	13.31	0.00	***

*** $P<0.001$; ** $P<0.01$; * $P<0.05$; $^+$ $P<0.1$

Furthermore, physiological measurements revealed that distant populations of wild *C. arabica* differentiated in their ecophysiological behavior over seasons, as shown by significant interactions of season and site ($P < 0.001$; Table 8). Comparison of transpiration rates (E) for the populations showed Harenna and Bonga to have slightly lower E in contrast to the other two sites in the wet season and a controversial relationship was found during dry season with a trend of higher E for Harenna and Bonga than for Berhane-Kontir and Yayu in period of reduced water availability.

Despite this trend of higher transpiration rates and stomatal conductance during dry season, the Harenna populations consistently had the highest water-use efficiency (WUE_i) of all populations compared with most profound differences during dry season. Therefore, differences in WUE_i were mainly due to the maintenance of a high net photosynthetic rate, which was less reduced in Harenna and Bonga provenances during the dry period.

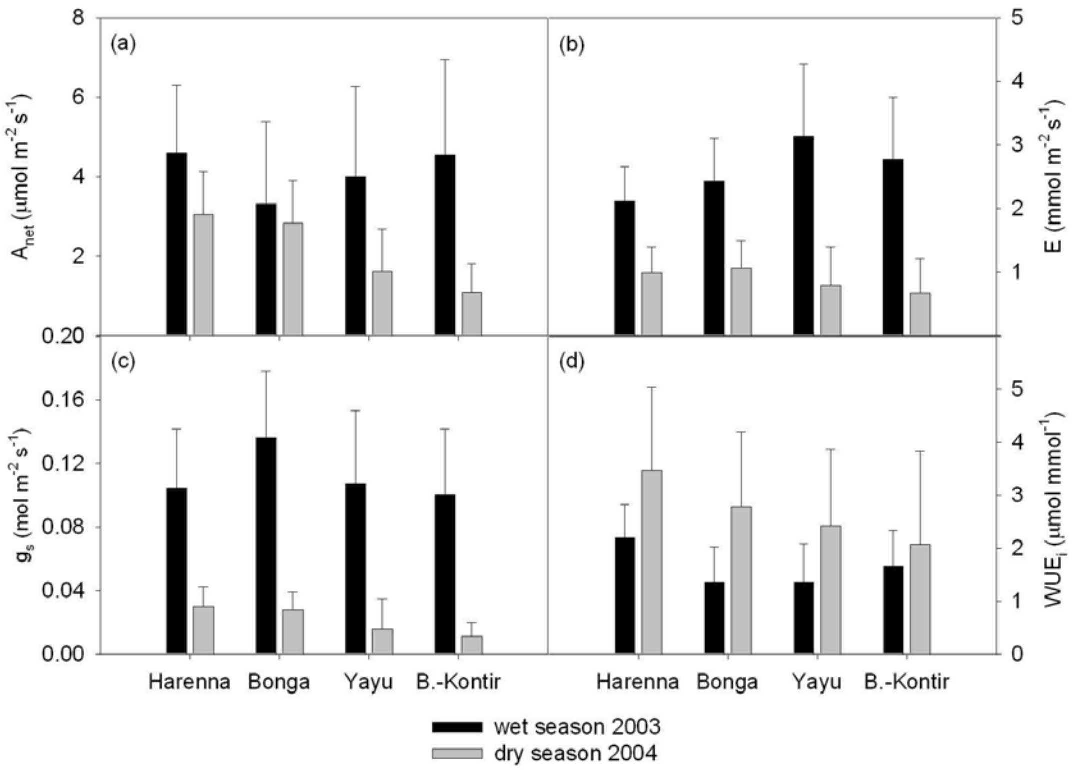

Figure 10: Seasonal changes in midday gas exchange parameters in wild *C. arabica* populations from different habitats measured *in-situ*; (a) net photosynthetic rate (A_{net}, µmol m^{-2} s^{-1}), (b) transpiration rate (E, mmol m^{-2} s^{-1}), (c) stomatal conductance (g_s, mol m^{-2} s^{-1}) and (d) instantaneous water-use efficiency (WUE_i, µmol $mmol^{-1}$)

The diurnal course of *in-situ* gas exchange (Figure 11) showed no clear differences among the provenances of the study sites. Net photosynthetic rates (A_{net}) started at high level in the morning and decreased either around noon (Berhane-Kontir) or in the afternoon (Harenna, Bonga, Yayu). Transpiration rates (E) reached their maximum in the late morning or at midday, respectively, and were slightly lowered at the rest of the day. With regard to stomatal behavior, diurnal variation in leaf conductance (g_s) was characterized by strong midday depression with a comparably high level in the morning, followed by steep decreases and stomatal closure before midday with no fully recovery in the later afternoon. The calculated instantaneous water-use efficiency (WUE_i) in Bonga and Yayu did not follow a striking diurnal pattern, Harenna and Berhane-Kontir however, showed to change over the day exhibiting steep increases in WUE_i in the afternoon. Hence, population differences in WUE_i were mainly due to their specific ability in reducing transpirational water loss rather than their capability to maintain net photosynthesis during periods of reduced water availability.

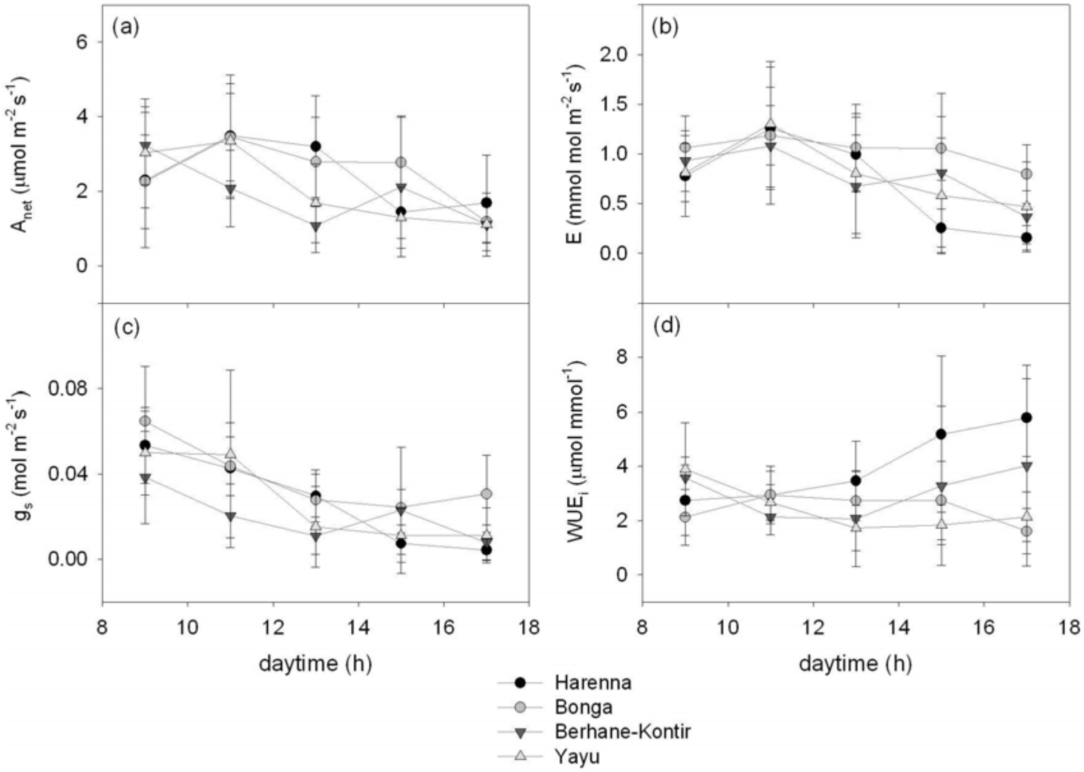

Figure 11: Diurnal parameters of gas exchange in wild *C. arabica* populations from different habitats measured *in-situ*; (a) net photosynthetic rate (A_{net}, µmol m^{-2} s^{-1}), (b) transpiration rate (E, mmol m^{-2} s^{-1}), (c) stomatal conductance (g_s, mol m^{-2} s^{-1}) and (d) instantaneous water-use efficiency (WUE_i=A_{net}/E, µmol $mmol^{-1}$) during dry period.

In order to fully understand the variation in ecophysiological behavior of the coffee plants found in this study, the dependency of populations' gas exchange activity on the specific atmospheric conditions was analyzed (Figure 12). As shown in Figure 12 a, there was a robust association between rates of net photosynthesis (A_{net}) and stomatal conductance (g_s) of individual leaves of all habitats that was best described by a logarithmic function (r^2=0.63).

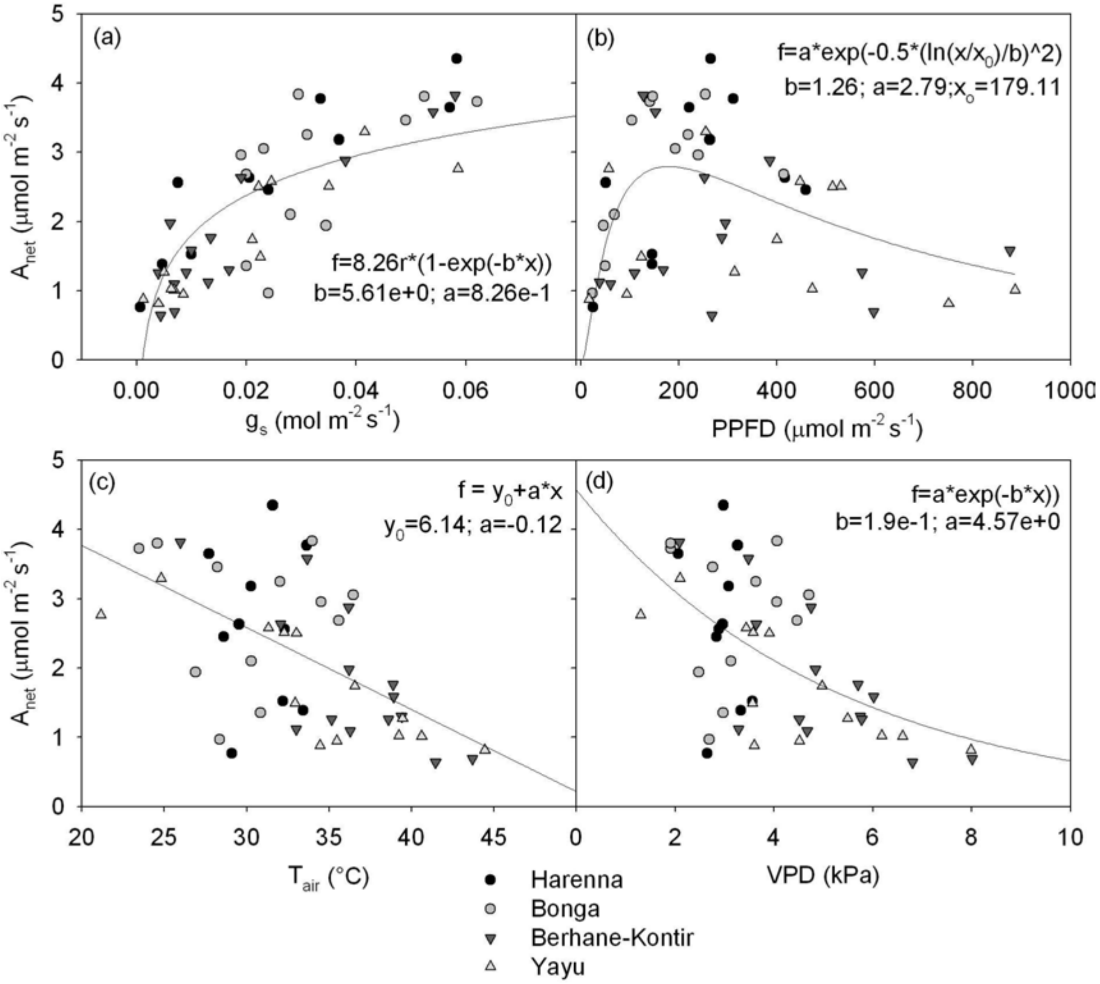

Figure 12: Changes in net photosynthetic rate (A_{net}) as a function of (a) stomatal conductance (g_s, mol m^{-2} s^{-1}), (b) photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}), (c) air temperature (T_{air}, °C) and (d) leaf-to-air vapor-pressure deficit (VPD, kPa) in wild *C. arabica* populations from different habitats.

Results revealed that assimilation was substantially reduced because of stomatal limitations when g_s dropped below 0.02 mol m^{-2} s^{-1}, although non-stomatal effects on A_{net} could not be ruled out as shown by varying photosynthesis under changing conditions related to light availability (Figure 12 b). In fact, a response pattern of increasing maximum photosynthetic rates with increasing light up to low light levels ($PPFD_{sat}$ = 179) was found, followed by a decline in A_{net} at further increases in light intensities. Also, air temperature (T_{air}; $r^2=0.32$) as well as leaf-to-air vapor pressure deficit (VPD; $r^2=0.33$) were negatively related to rates of A_{net} (Figure 12 c,d). Comparable high values of A_{net} were found up to temperature levels of 35°C and 4 kPa, respectively; however an increase in both parameters resulted in further depression in carbon assimilation rate.

Furthermore, a strong relationship between stomatal conductance and air temperature (T_{air}) as well as vapor-pressure deficit (VPD) was found (Figure 13). It showed to be closely negatively related to air temperature (T_{air}; $r=0.55$, $P<0.01$) and leaf-to-air water vapor pressure deficit (VPD; $r=0.53$, $P<0.01$). Highest stomatal conductance was found at T_{air} of around 25°C, which represents the lowest *in-situ* temperature levels measured in this study and linearly decreased with further steps of temperature increases, while a non-linear function fit best to describe the decrease in g_s with increases in VPD (kPa) with values 2 kPa being best for maximum stomatal conductance of the plants.

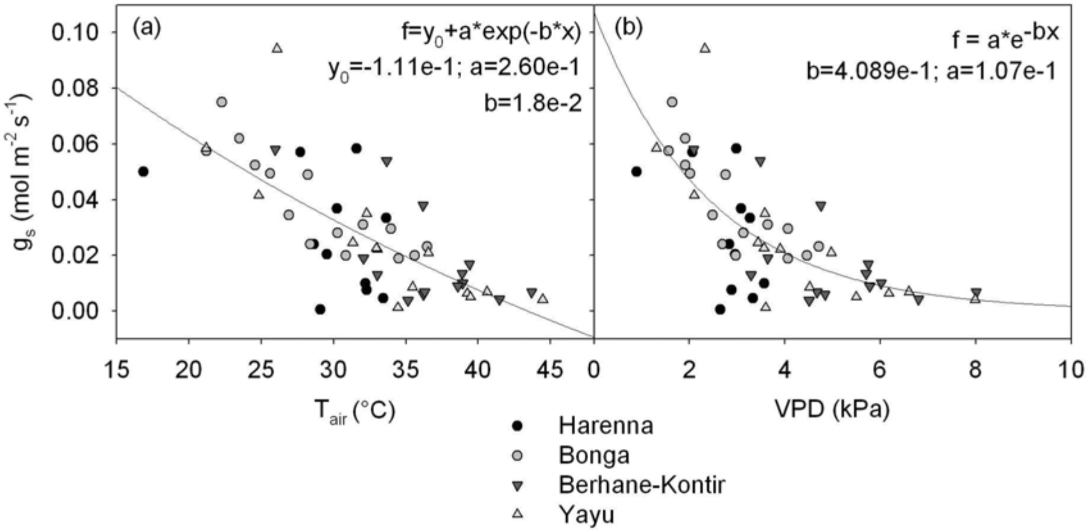

Figure 13: Changes in stomatal conductance (g_s, mol m^{-2} s^{-1}) as a function of (a) air temperature (T_{air}, °C) and (b) leaf-to-air vapor-pressure deficit (VPD, kPa) in the wild *C. arabica* populations from different habitats.

Additionally, habitat specific responses in ecophysiology to environmental variables are shown in Table 9. Results showed that responses were highly depending on specific environmental conditions when measurements were taken. For Harenna and Bonga provenances, light intensity measured as photosynthetic photon flux density (PPFD) had a significant positive influence on net photosynthesis (A_{net}), whereas diurnal changes in A_{net} in plants originating from forests of Berhane-Kontir and Yayu were more dependent on variations in air temperature (T_{air}) and vapor-pressure deficit (VPD) rather than fluctuations in PPFD. Transpiration rates however, did not show to be significantly depending on all measured surrounding environmental conditions, while stomatal conductance was negatively correlated with T_{air} and VPD in Bonga, Berhane-Kontir and Yayu.

Table 9: Correlation coefficients (r) between physiological and environmental parameters (PPFD = photosynthetic photon flux density, T_{air} = air temperature, VPD = vapor pressure deficit) of wild *C. arabica* populations from contrasting habitats measured *in-situ* during dry season in 2004. All regressions were linear. Significant correlations are printed in bold letters.

Parameter		PPFD	T_{air}	VPD
Net photosynthetic rate	Harenna	**0.45**	0.28	0.22
	Bonga	**0.49**	0.23	0.28
	Yayu	0.02	**-0.60**	**-0.55**
	Berhane-Kontir	0.02	**-0.55**	**-0.52**
Transpiration rate	Harenna	0.37	0.13	0.11
	Bonga	0.29	0.05	0.12
	Yayu	-0.02	-0.36	-0.33
	Berhane-Kontir	0.05	-0.23	-0.24
Stomatal conductance	Harenna	0.18	-0.35	-0.40
	Bonga	-0.20	**-0.68**	**-0.62**
	Yayu	-0.15	**-0.68**	**-0.61**
	Berhane-Kontir	-0.13	**-0.57**	**-0.55**

4.2.4 Carbon isotope discrimination

Carbon stable isotope signatures of plant foliage as an indicator of long-term WUE ($\delta^{13}C$) of distant *C. arabica* were significantly affected by seasonal variation in moisture availability (Figure 14 a). During wet season plants displayed low $\delta^{13}C$ values, while having high $\delta^{13}C$ in poor rainfall seasons. Furthermore, results of $\delta^{13}C$ showed that intrapopulation variance of carbon isotope discrimination correspond to the degree of spatio-temporal heterogeneity of water availability of the distant habitats. Specifically, a negative correlation existed between total annual precipitation and foliar $\delta^{13}C$ among distant habitats. However, population variation was less profound in wet season than under drought conditions. In addition, carbon isotope analysis of the stem material (Figure 14 b), a trait that is believed to show high heritability, exhibited a similar pattern though not being significant. However, results revealed the tendency of Harenna provenances to exhibit higher values than the more south-western coffee populations. Interestingly, Berhane-Kontir showed a high intrapopulation variation in wood $\delta^{13}C$ in contrast to other provenances examined.

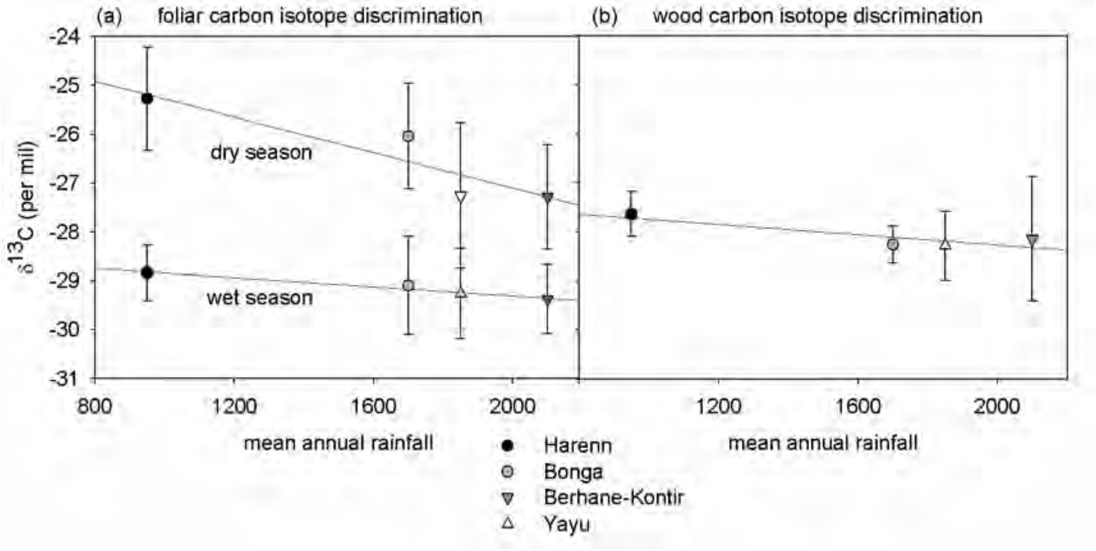

Figure 14: Relationship between carbon isotope discrimination ($\delta^{13}C$) and total annual precipitation among wild C. arabica populations from different habitats; (a) foliar $\delta^{13}C$ and (b) wood $\delta^{13}C$.

The results further support that integrated WUE as measured by $\delta^{13}C$ signatures reflects population differences similar to instantaneous water-use efficiency (A_{net}/E, WUE_i) as shown in Figure 15. Both the gas exchange and carbon isotope data indicated that the trees responded to declining moisture availability by increasing WUE.

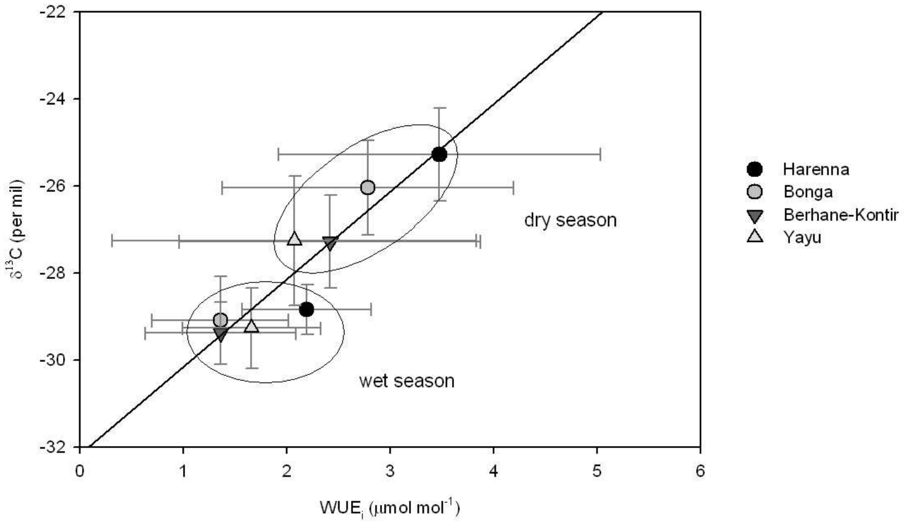

Figure 15: Relationship between instantaneous water-use efficiency (WUE_i) obtained from gas exchange measurements and long-term water-use efficiency ($\delta^{13}C$) taken from carbon isotope discrimination measures of wild *C. arabica* populations from different habitats.

4.3 *Ex-situ* study: Ecophysiological response to drought stress in wild *Coffea arabica* populations under common-environment conditions

4.3.1 Environmental conditions

The treatment combinations with two light regimes (shade, open sun) and two watering levels (well watered, drought-stressed) resulted in contrasting environmental conditions (Figure 16). The contrasting water regimes applied to coffee seedlings significantly influenced soil moisture contents, which ranged from near field capacity to severe soil desiccation (Figure 16 a). Whereas well watered seedlings maintained a soil water content of approximately 43.01 % (±2.82) throughout the experiment independent of the influence of light availability, soil moisture levels decreased significantly in the non-irrigated treatments. During the first five days after treatment start, withholding water

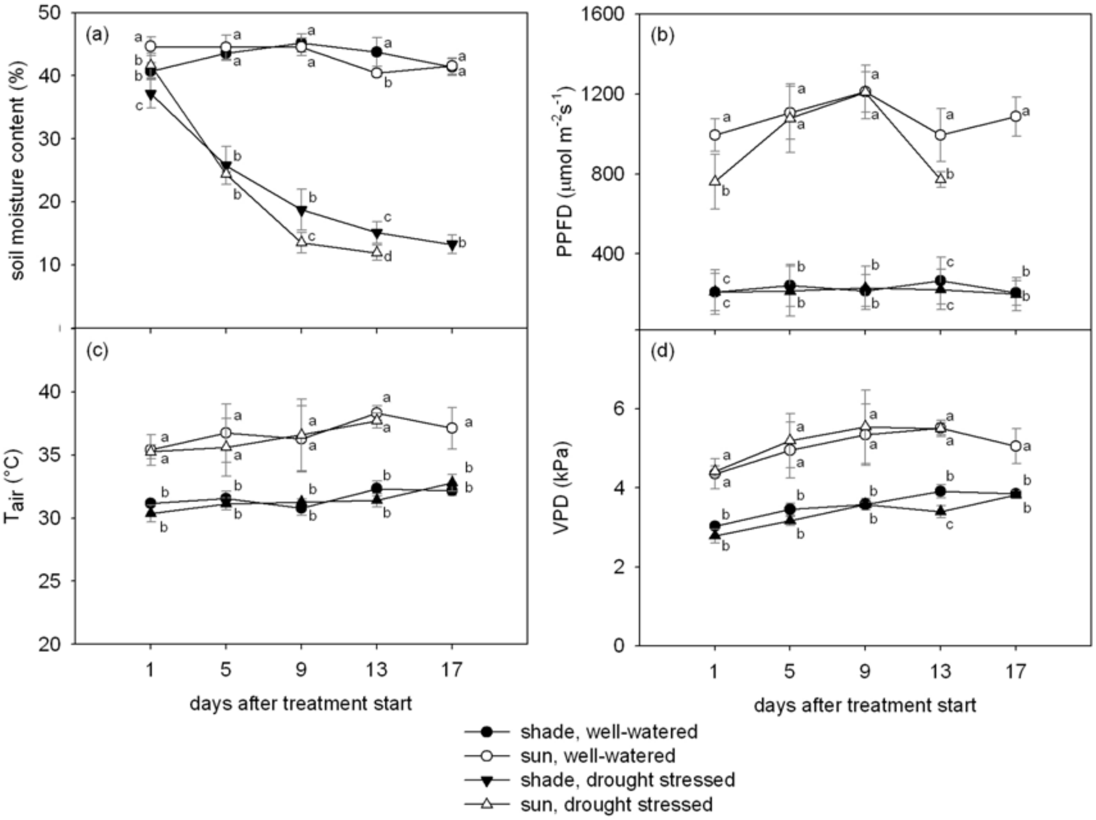

Figure 16: Changes in (a) predawn soil moisture content (%), (b) photosynthetic photon flux density (PPFD, µmol photons m^{-2} s^{-1}), (c) air temperature (T_{air}, °C) and (d) leaf-to-air vapor pressure deficit (VPD, kPa) in four treatments of the *ex-situ* experiment. Symbols indicate daily mean ± SD; where no error bars are apparent, they are smaller than the size of the symbol; n = 24.

resulted in a strong decrease in soil water availability, irrespective of the influence of light availability. After that a significant interaction between irrigation level and light availability was detected ($P<0.05$, ANOVA), indicating that shade and open sun environments differed with regard to soil drying, with a faster drying in pots under open sun compared to shade grown seedlings. However, at the end of the experimental period, soil moisture levels decreased to 13.31 % under shade and 11.97 % under open sun. The interaction of habitat and watering level was not significant, and thus there were no differences in the rate of soil water extraction among the populations from different habitats. In addition, the two irradiance levels resulted in contrasting atmospheric conditions; plants exposed to high irradiance levels had to cope with higher values of photosynthetic photon flux density (PPFD, Figure 16 b) and higher air temperatures (T_{air}, Figure 16 c). Furthermore, high temperatures due to open sun exposure provoked high leaf-to-air vapor pressure deficits (VPD, Figure 16 d) compared to shade conditions.

The diurnal variation of environmental parameters also changed depending on the respective treatment combination (Figure 17). There were contrasting values of mean maximum PPFD levels observed at around 12 pm, which reached approximately 440 µmol m^{-2} s^{-1} in the shaded treatments, while plants grown in open sun experienced light intensities of about 1600 µmol m^{-2} s^{-1}. Hence, light availability under shade was reduced by 75 % compared to open sun conditions. Under shade, high variability in PPFD values resulted from the occurrence of sunflecks allowed by the shade technique resembling natural habitat conditions of field-grown plants. Light availability contrasts among the treatments were also accompanied by profound differences in air temperatures (T_{air}), which reached a daily maximum at about 2 pm. Specifically, plants in the full light treatment experienced a broad range of temperatures across the day fluctuating from 27 to 41°C, while a lower and rather narrow temperature range of 22 to 26°C was found under shade conditions. A similar trend was observed for leaf-to-air vapor pressure deficits (VPD, kPa). In the shade environments, plants were exposed to significantly lower VPD in contrast to those in the sun treatments. The morning values of VPD under shade were about 1.8 kPa and reached a maximum of 4.4 kPa at around 2 pm, while exposure to open sun led to VPD values varying from 2.7 to 6.4 kPa through the course of the day with the highest increase around midday.

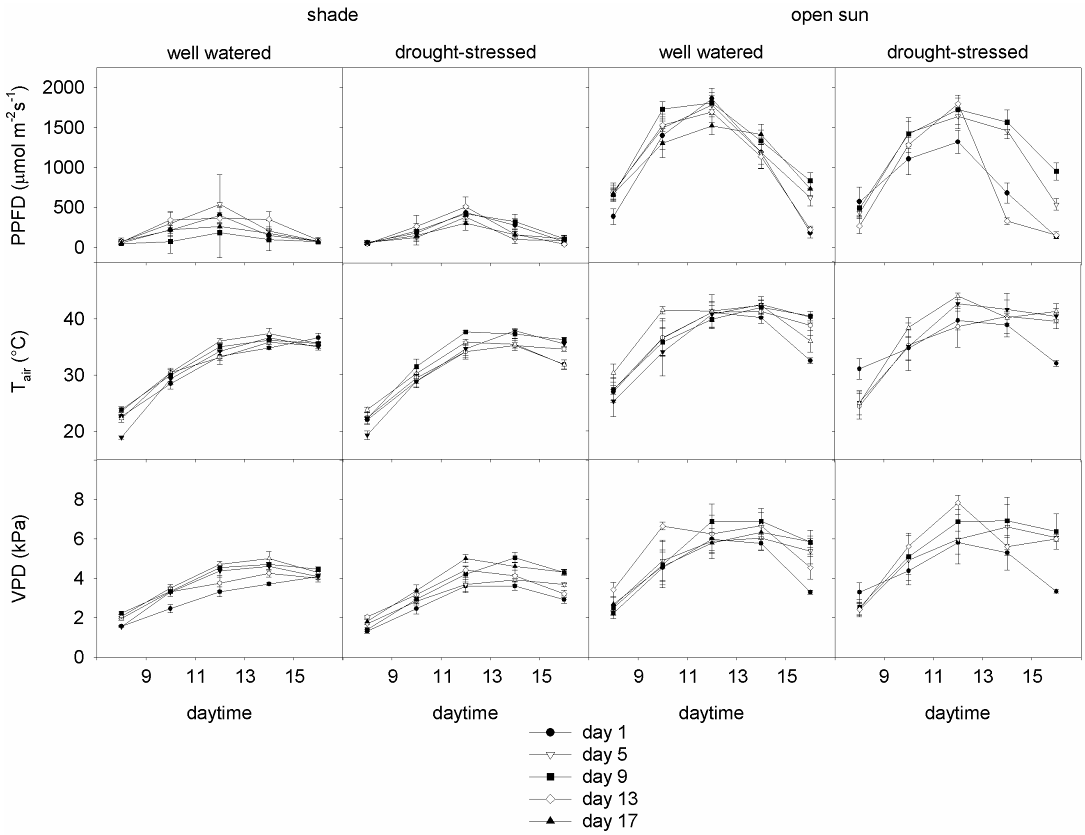

Figure 17: Diurnal changes in photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}), air temperature (T_{air}, °C) and leaf-to-air vapor pressure difference (VPD, kPa) in the four different treatments of the *ex-situ* experiment (a) shade, well watered; (b) shade, drought-stressed; (c) open sun, well watered and (d) open sun, drought-stressed on five days. Each value is the mean ± SD of 24 single measurements.

4.3.2 Leaf water relations

The influence of contrasting light and watering intensities resulted in a range of leaf water relations. Time and habitat differences were interpreted in the context of different tissue water relations within each treatment (Table 10). Because of a limited sample size only differences among habitats are presented. An increase in soil moisture deficit in the two non-irrigated treatments (shade, drought-stressed; open sun, drought-stressed) was followed by highly significant time-induced changes effects ($P<0.001$) for all leaf water characteristics. Significant time effects in the repeated ANOVA model were also found for some parameters in the treatments with a consistently sufficient moisture supply (shade, well watered; open sun, well watered) due to differences in microclimate on the specific measurements days.

Table 10: F-ratios from repeated analyses of variance comparing leaf water relation parameters in wild *C. arabica* populations from different habitats. Differences between habitats over time were tested separately for each treatment combination.

Treatment	Parameter	Time		Time*habitat	
Shade, well watered	leaf water content $_{\text{predawn}}$	2.25	+	2.07	
	osmotic potential $_{\text{midday}}$	2.93	*	0.47	
	osmotic potential $_{\text{predawn}}$	9.64	***	0.61	
	water potential $_{\text{midday}}$	15.72	***	0.57	
	water potential $_{\text{predawn}}$	15.49	***	1.05	
Shade, drought-stressed	leaf water content $_{\text{predawn}}$	11.44	***	1.87	+
	osmotic potential $_{\text{midday}}$	22.56	***	2.61	*
	osmotic potential $_{\text{predawn}}$	13.12	***	1.60	
	water potential $_{\text{midday}}$	65.31	***	4.67	***
	water potential $_{\text{predawn}}$	45.05	***	2.64	*
Open sun, well watered	leaf water content $_{\text{predawn}}$	3.99	*	0.69	
	osmotic potential $_{\text{midday}}$	2.03		0.60	
	osmotic potential $_{\text{predawn}}$	14.19	***	1.54	
	water potential $_{\text{midday}}$	12.44	***	2.17	*
	water potential $_{\text{predawn}}$	14.21	***	1.67	
Open sun, drought-stressed	leaf water content $_{\text{predawn}}$	39.29	***	5.13	**
	osmotic potential $_{\text{midday}}$	44.66	***	3.91	**
	osmotic potential $_{\text{predawn}}$	64.11	***	4.87	**
	water potential $_{\text{midday}}$	75.18	***	1.66	
	water potential $_{\text{predawn}}$	94.95	***	0.75	

*** P<0.001; ** P<0.01; * P<0.05; $^{+}$ P<0.1

Additionally no significant interactions of time and habitat for almost all parameters in the irrigation treatments were revealed; hence differences in behavior among the populations from different habitats were not detected. The only exception was the leaf water potential at midday, and the plants from the four habitats differentiated in this respect in the well watered, open sun treatments. However, habitat differences became more apparent when water was withheld. Drought-stressed plants under shade showed a significant habitat*time effect for midday leaf osmotic potential as well as for leaf water potential at predawn and midday, while withholding water from plants grown under open sun resulted in significant differences among habitats for predawn and midday leaf osmotic potential and leaf water content.

The habitat-specific drought responses in plant leaf water status over the experimental period showed that soil drying period had a significant effect on the development of relative leaf water content (RWC) under shade and open sun environments (Figure 18). At the commencement of the treatments, all plants were still under conditions of sufficient water supply and exhibited leaf water contents of about 0.78 (± 0.01) under both light regimes. Despite the following progress of progressed water deficit, all plants showed to be rather unresponsive to variations in soil moisture conditions and maintained stable RWC over a range of soil moisture conditions. Only when severe soil drying reached moisture contents of less than 20 %, leaf water content declined sharply under both light availability treatments. Only at the end of the soil drying period, plants from distant habitats differentiated in the degree of stress response, with Harenna being more strongly affected ($P<0.1$ under shade; $P<0.05$ under open sun). Loss in RWC reached 0.56 % in Harenna, while the plants from the south-western habitats exhibited minimum values of more than 60 %.

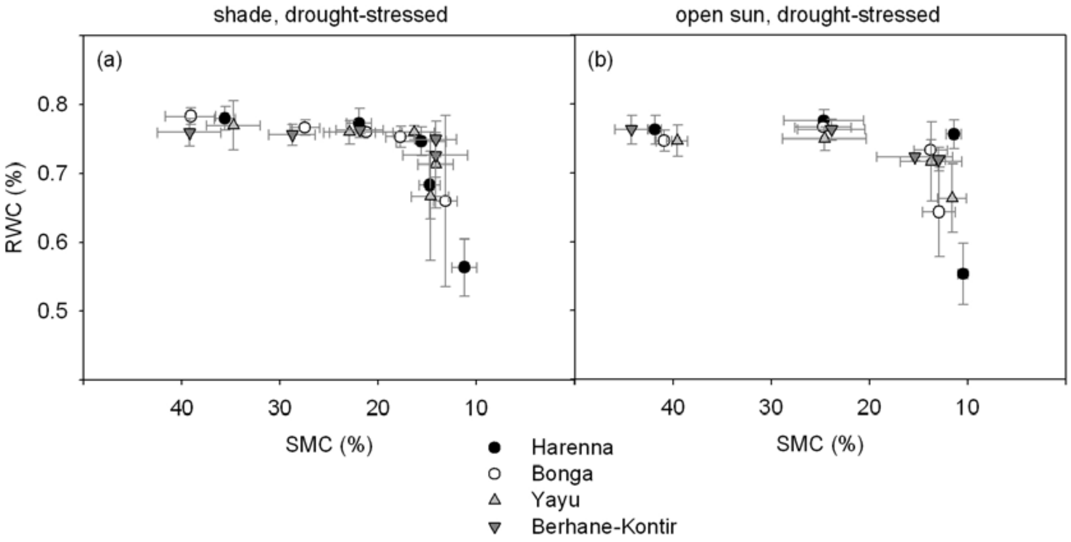

Figure 18: Changes in predawn leaf water content (%) in wild *C. arabica* populations from different habitats in (a) shade and (b) open sun over a period of soil drying. Symbols indicate mean ± SD; n = 3.

While both, predawn and midday leaf water potential values (Ψ_{pd}, Ψ_{md}) remained relatively constant in irrigated treatments for all populations (Ψ_{pd}: -0.27±0.07 MPa; Ψ_{md}: -1.13±0.10 MPa; data not shown), varying soil moisture conditions under drought-stressed environments influenced leaf water status of all populations (Table 10; Figure 19). All populations exhibited consistently lower midday Ψ values compared with predawn over the whole soil drying period, and both parameters were curvilinear related to soil water content, independent of light intensity. After withholding irrigation plants maintained Ψ_{pd} values close to zero over a range of soil moisture contents. However, where progressed

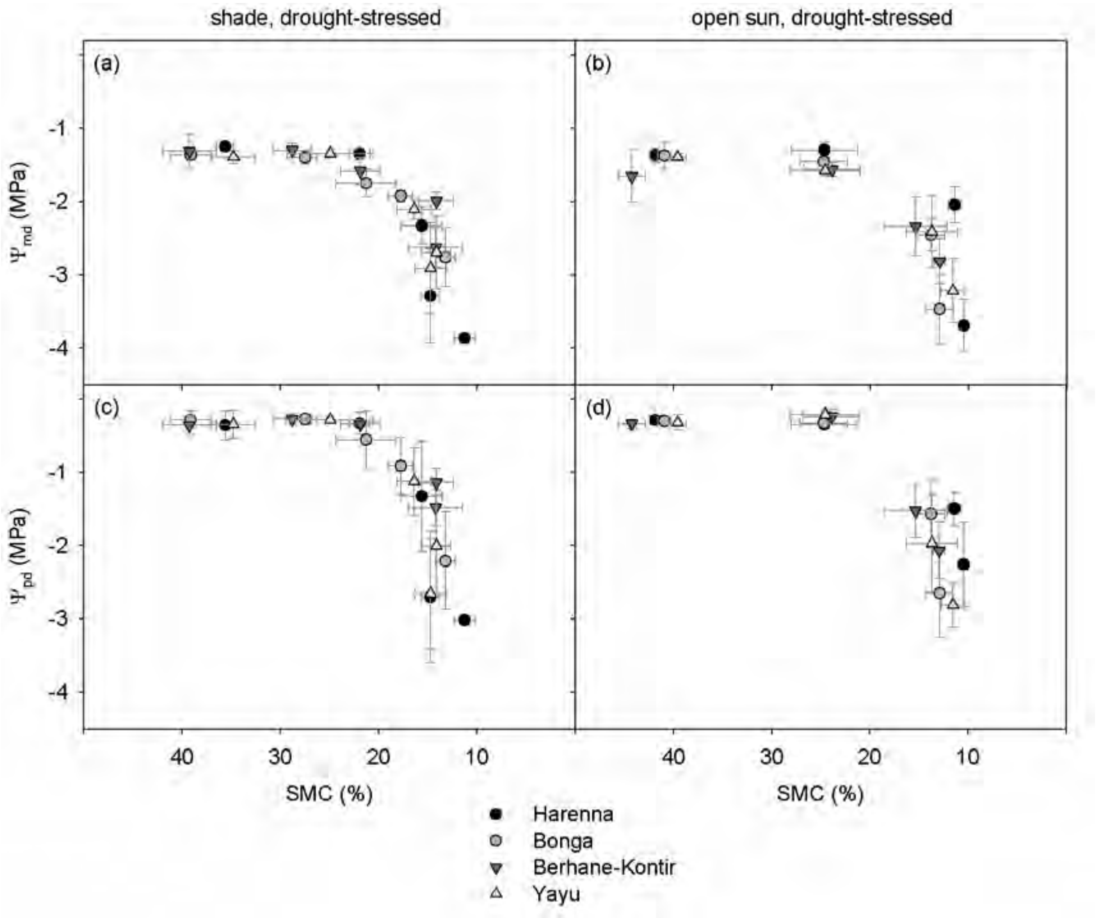

Figure 19: Changes in leaf water potential (ψ) in wild *C. arabica* from different habitats over a period of soil drying; midday leaf water potential under shade (a) and in open sun (b); predawn leaf water potential in shade (c) and in open sun. Bars indicate ± SD; n = 3.

soil drying led to soil moisture contents of less than 20 %, the Ψ_{pd} and Ψ_{md} values of all populations significantly decreased and reached minimum values until the end of the experiment. For populations from the different habitats, differences in responses to soil drying were not detected at the beginning of the soil drying period, but became marked when plants were seriously affected by reduced soil water availability. Harenna populations were highly stressed, reaching very low leaf water potentials followed by Yayu and Bonga, whereas Ψ_{pd} and Ψ_{md} values decreased to a lesser degree in Berhane-Kontir.

The soil drying period also influenced plant behavior with respect to leaf osmotic potential (π, Figure 20). Despite gradual depletion of soil water in the first days of the experiment, no significant treatment-induced changes in π values at predawn and midday were detected, and maximum values were maintained at approximately -1.5 MPa.

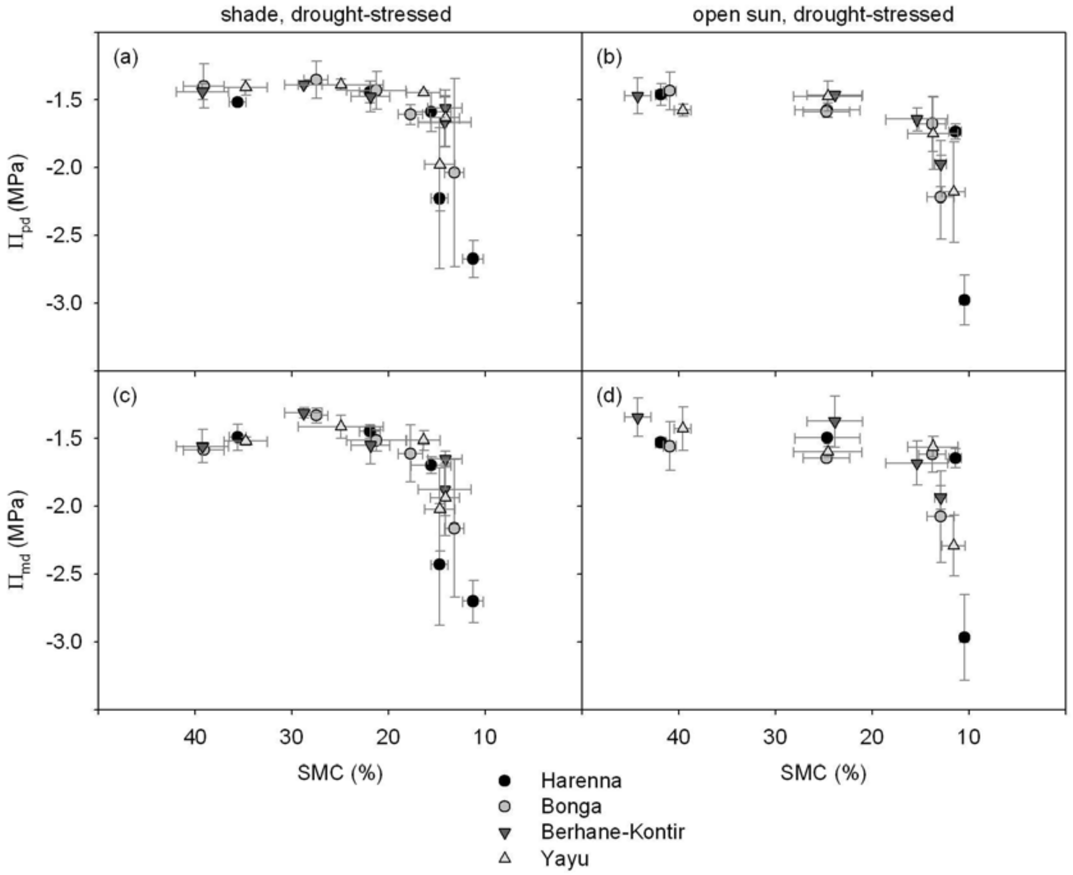

Figure 20: Changes in leaf osmotic potentials (π) in wild *C. arabica* populations from different habitats over a period of soil drying; predawn leaf osmotic potential under shade (a) and in open sun (b); midday leaf osmotic potential under shade (c) and in open sun (d). Bars indicate mean ± SD; n = 3.

Further decreases in soil moisture content to less than 20 %, however, resulted in a strong decline of both parameters at the end of the observation period. At this stage of the experiment, differences in π values among populations from the four habitats were detected. Specifically, Harenna and Berhane-Kontir exhibited the most contrasting pattern in π_{md} as well as in π_{pd}, while Yayu and Bonga showed intermediate degree of stress response.

In order to emphasize habitat differences, the severity of the internal drought stress experienced by the distant populations was monitored by calculating a drought-susceptible index (*S*) for selected tissue water relations (Figure 21). Populations from the four habitats clearly differentiated in their degree of stress response at the end of the drought-stress period, indicating that the populations exhibited a contrasting resistance to drought stress. While the physiological performance in the Harenna populations was strongly affected by soil drought irrespective of the specific light intensity and showed a high *S*, seedlings from Berhane-Kontir showed a good drought performance, revealed by a low *S* in relative leaf water content, predawn leaf water potential and leaf osmotic potential. The Bonga and Yayu populations, however, showed intermediate drought stress response.

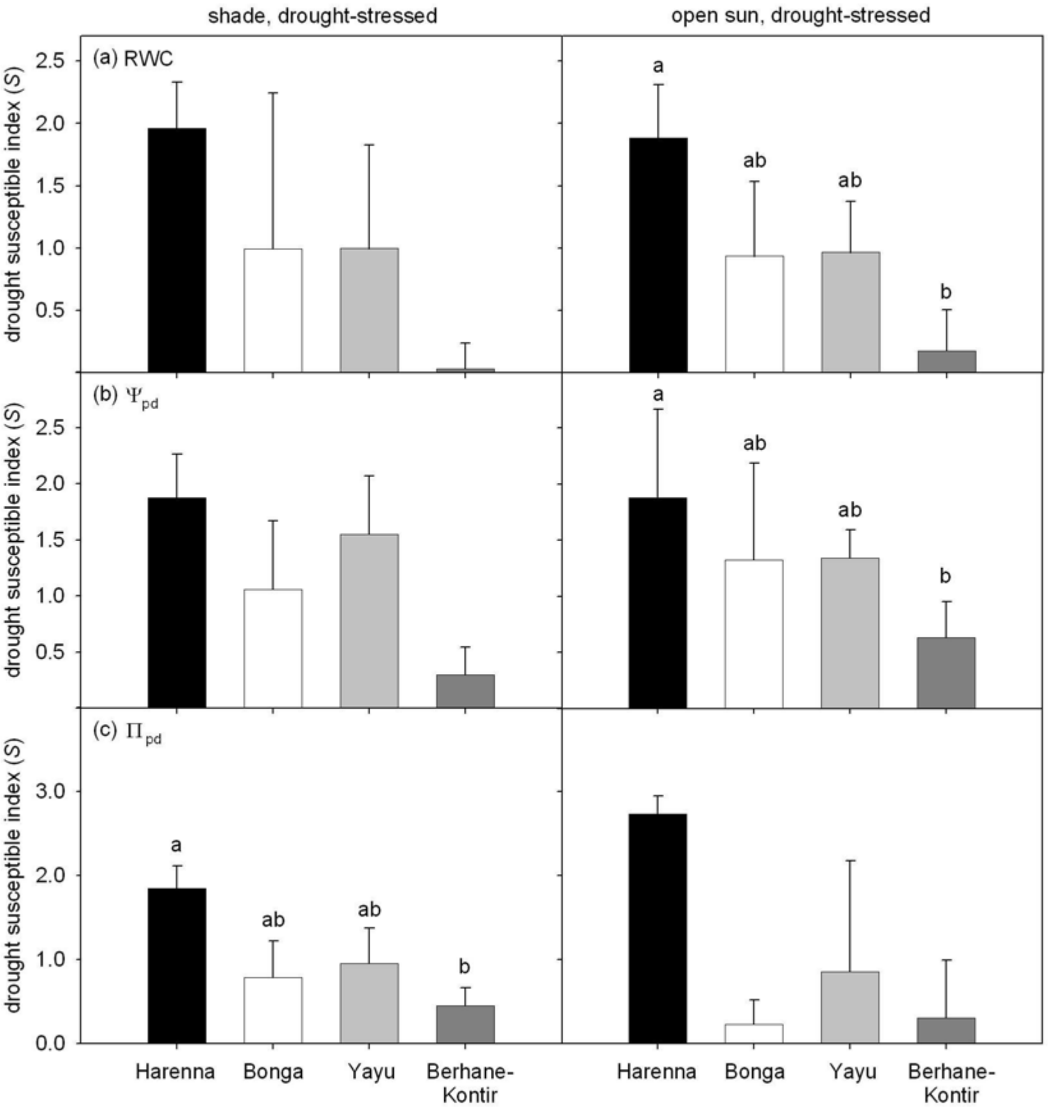

Figure 21: Drought susceptible index (*S*) of leaf water relation parameters; (a) relative water content (RWC), (b) predawn water potential (Ψ_{pd}) and (c) predawn osmotic potential (π_{pd}) of wild *C. arabica* populations from different habitats at the end of drought stress period grown under shade (left panel, after 17 days) and open sun (right panel, after 13 days), respectively. Bars indicate mean ± SD; n = 3.

The interrelations of relative leaf water content, osmotic and turgor potential of the leaves in response to a variation in leaf water potential showed populations' behavior regarding their control of tissue water relations (Figure 22). Because habitat-specific differences in the relationship of the leaf water status parameters were not revealed, data is presented for all populations irrespective of their origin. Under both shade and open sun conditions, a strong negative relationship was found for turgor potential (*P*) that declined sharply over the whole period of stress imposition. Relative water content (RWC_{pd}) and osmotic potential (π_{pd}) of leaves varied also in a highly predictable fashion in relation to leaf Ψ_{pd}. However, these parameters were maintained at considerable high level at an early stage of the experiment and decreased only after a Ψ_{pd} value of about -2 MPa was reached. Though osmotic potential decreased at lowered water potential, it was not accompanied by maintenance of turgor potential. At the end of the experiment, the lethal values for osmotic potential were <2 MPa, <0.6 for RWC and -2.5 MPa for leaf water potential.

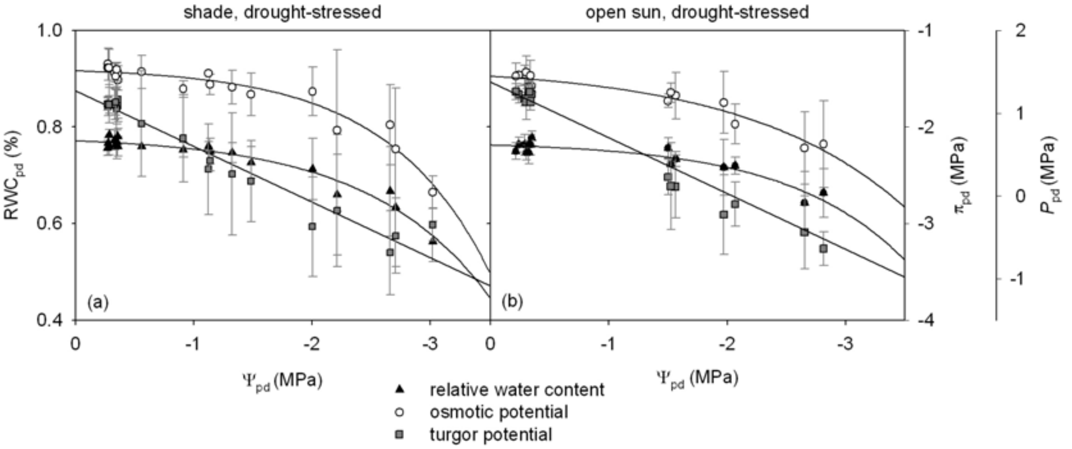

Figure 22: Relative leaf water content (RWC_{pd}; %), osmotic (π_{pd}; MPa) and turgor potential (P_{pd}; MPa) measured at predawn as a function of predawn leaf water potential (Ψ_{pd}, MPa) in wild *C. arabica* populations grown under shade (a) and open sun (b) over a drought stress period.

Over the period of reduced soil moisture availability, wild *C. arabica* seedlings were able to recover from midday tissue desiccation, which was reflected by the differences in midday and predawn leaf water potential ($\Delta\ \Psi_{md-pd}$; Figure 23). In general, the diurnal range of dynamics in leaf water potential characteristics was slightly lower under shade than under open sun conditions. Under shade, plants maintained the ability to control the extent of their daily decline $\Delta\ \Psi_{md-pd}$ over the whole experiment time with slight decreases under severe soil drought, while under full irradiance; there was a gradual decrease over the whole range of soil moisture conditions.

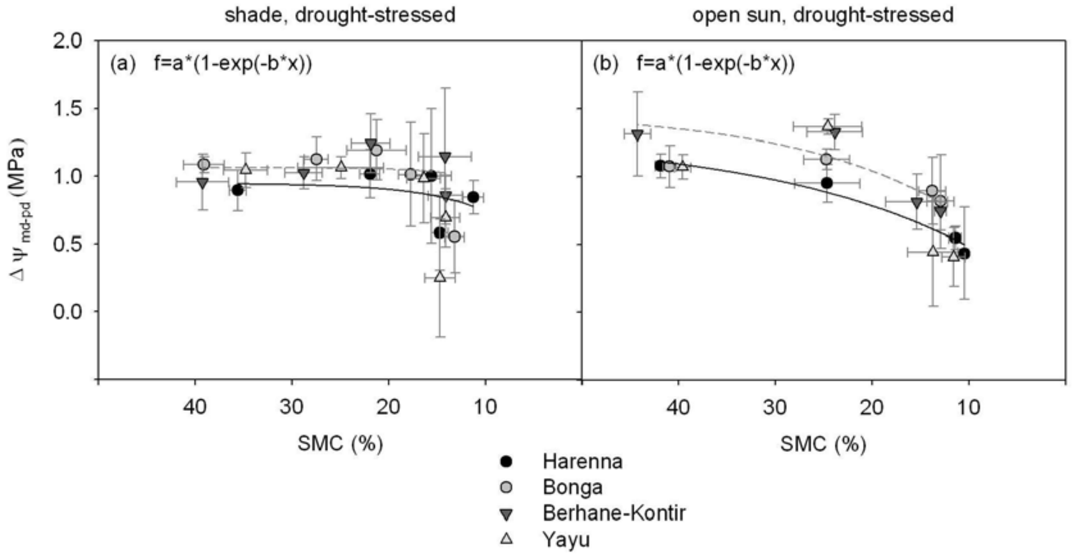

Figure 23: **Influence of soil water content (%) on the difference in predawn and midday leaf water potential ($\Delta\ \Psi_{pd\text{-}md}$; MPa) as an estimate for overnight recovery from desiccation stress wild *C. arabica* populations from different habitats grown under shade (a) and open sun (b) measured over a period of soil drying. Regression lines represent the populations from most contrasting habitats (– Harenna; -- Berhane-Kontir).**

Additionally, fluctuations in leaf water potential between midday and predawn measurements revealed a contrasting behavior among seedlings of distant populations with most striking differences among plants originating from Harenna and Berhane-Kontir. In the shade and drought stress treatment, Harenna populations exhibited closely related midday and predawn values in leaf water potential with a narrow range of $\Delta\ \Psi_{pd\text{-}md}$ MPa, whereas Berhane-Kontir seedlings maintained the highest diurnal range.

The comparison of the midday and predawn measurements of the leaf osmotic potentials (π) showed decreasing values over the whole period of soil drying under the two light availability treatments (Figure 24). Their tendency towards the bisecting line however, revealed that both midday and predawn estimates decreased to similar extent, hence no clear diurnal pattern became obvious. Furthermore, there were no differences among populations from distant habitats in this respect, despite the π of the Harenna populations being heavily aggravated at the end of the experimental period in contrast to the seedlings originating from the other locations.

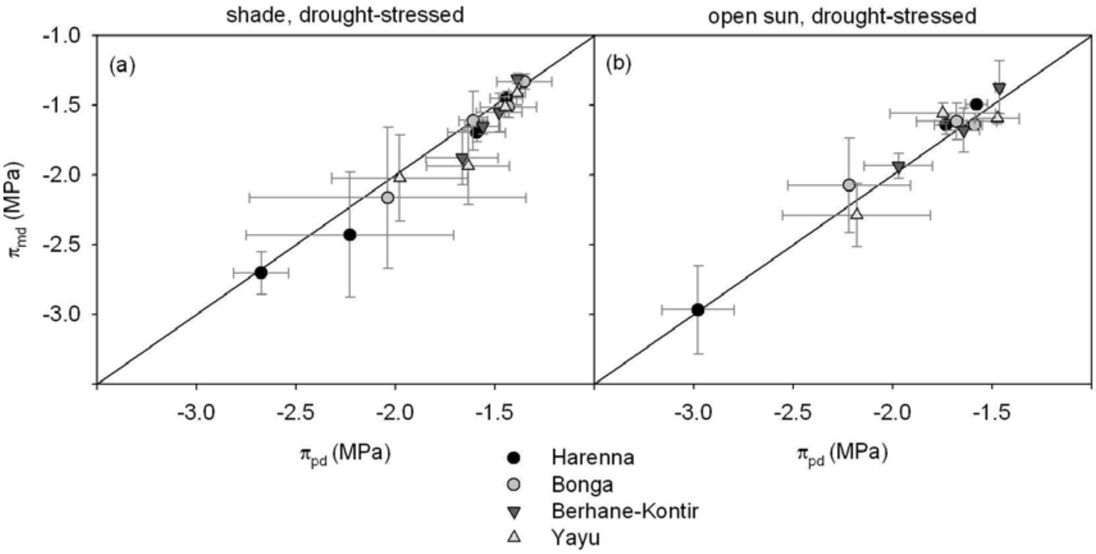

Figure 24: **Relationship between predawn (pd) and midday (md) measurements of leaf osmotic potential (π) in wild *C. arabica* populations from different habitats grown under shade (a) and open sun (b) measured over a period of soil drying.**

4.3.3 Gas exchange measurements

Daily changes in gas exchange activity

The contrasting light and water availability treatments created a range of environmental conditions that resulted in different influences on the mean daily values of gas exchange activity of the selected habitats and populations (Table 11). Differences between habitats and populations nested within habitats over time were tested separately for each treatment combination. Time effects were highly significant ($P < 0.001$) or at least significant ($P < 0.01$) for all plant parameters in the four different treatments. There were also significant time*habitat effects for most characters in the water-deficit environments, except for water-use efficiency (WUE_i) in the open sun and drought-stressed treatment. Interaction of time and population (within habitat) was significant for most parameters under non-irrigated conditions and for a few in treatments with sufficient soil moisture supply.

Table 11: F-ratios from repeated ANOVA for mean daily gas exchange parameters in wild *C. arabica* populations from different habitats. Asterisks denote significant effects of time, time * habitat, time * population (nested within habitat).

Parameter	time		time*habitat		time*population (habitat)	
Shade, well watered						
A_{net}	20.64	***	1.18		2.44	**
E	41.95	***	2.35	*	2.05	*
g_s	15.59	***	2.53	*	2.19	**
WUE_i	14.55	***	1.20		0.77	
Shade, drought-stressed						
A_{net}	53.99	***	5.11	***	2.01	**
E	9.63	***	5.50	***	2.30	**
g_s	26.37	***	4.75	***	2.23	**
WUE_i	46.68	***	4.25	***	1.17	
Open sun, well watered						
A_{net}	53.99	***	1.75		1.46	
E	9.63	***	1.57	*	0.87	
g_s	26.37	***	1.01		1.07	
WUE_i	46.68	***	6.22	***	2.70	**
Open sun, drought-stressed						
A_{net}	218.26	***	7.54	***	2.47	**
E	180.65	***	7.23	***	1.20	
g_s	367.20	***	9.68	***	3.40	***
WUE_i	36.07	**	8.76	***	7.10	***

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; $^{+}P < 0.1$

The seedlings grown under shade with sufficient water supply showed a consistently high mean daily photosynthetic rate (A_{net}) throughout the experiment (Figure 25). Though no significant differences among the populations were revealed, there was a trend of higher net photosynthesis in the Harenna seedlings. Exposing irrigated plants to levels of high light intensity (Figure 25 b) led to a steady decline in the A_{net} rate to a minimum of 2 µmol m^{-2} s^{-1} nine days after start of the experiment. This sharp decrease in A_{net} for leaves exposed to open sun indicates that the *C. arabica* seedlings were sensitive to high light intensity after being shaded for nearly 6 months. By withholding water in the shade grown plants (Figure 25 c), the photosynthetic rate was maintained during the first week in all populations but steadily decreased with increasing drought stress during the drying cycle, whereas open sun conditions triggered a faster decrease in as soon as five days (Figure 25 d). However, both drought-stressed environments revealed different behavior

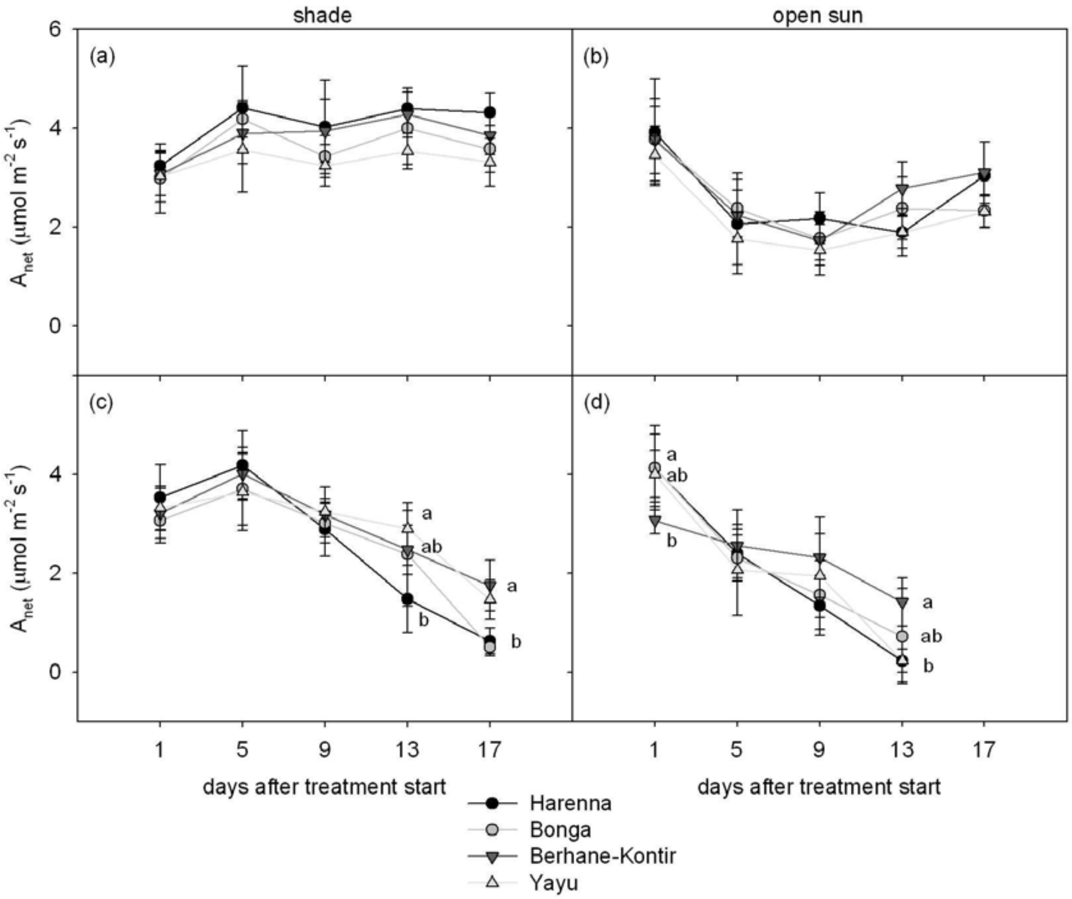

Figure 25: Mean daily net photosynthetic rate (A_{net}) of wild *C. arabica* populations from different habitats in treatment (a) shade, well watered; (b) shade, drought-stressed; (c) sun, well watered and (d) sun, drought-stressed over 17 days. Letters denote significant differences between habitats on specific days after treatment start (*P* < 0.05).

among the populations from different habitats, with a more rapid decrease in seedlings originating from the Harenna habitat reaching the lowest values at the end of the drought-stress cycle in contrast to Berhane-Kontir seedlings.

Transpiration rates (E) also changed according to treatment (Figure 26). Whereas E was kept at a high level around 1.18 to 2.43 mol m^{-2} s^{-1} remaining constant over time with a trend of higher values for seedlings from the Harenna populations in the shade and irrigation treatment (Figure 26 a), transpiration changed considerably in the other treatments. Under well watered conditions, high light intensities led to an initial transpiration rate of around 2 mol m^{-2} s^{-1}, followed by a strong decrease in the first week (Figure 26 b). After nine days however, values increased considerably and reached a similar level to that observed the beginning of the experiment. In the other treatments, E

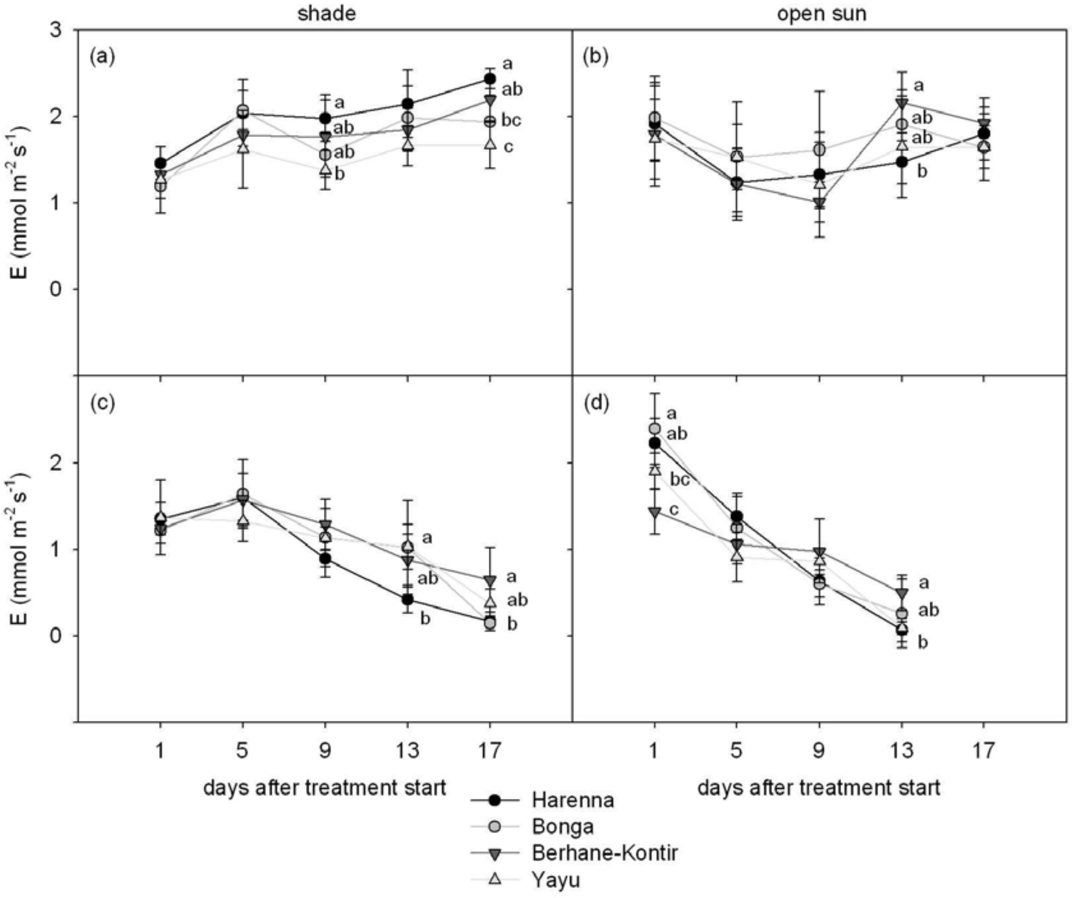

Figure 26: **Mean daily transpiration rate (E) of wild *C. arabica* populations from different habitats in treatment (a) shade, well watered; (b) shade, drought-stressed; (c) sun, well watered and (d) sun, drought-stressed over 17 days. Letters denote significant differences between habitats on specific days after treatment start (*P* < 0.05).**

decreased drastically when water was withheld and reached minimum values at the end of the experiment under shade as well as open sun (Figure 26 c, d). However, both soil drought treatments differed in the degree of declining transpiration rates depending on light intensity. At the beginning of the experiment, shade-grown plants were able to maintain stable transpiration rates and E of drought-stressed plants exposed to open sun started to diverge from that of the well watered plants only 4 days after withholding irrigation. Furthermore, behavior of the populations from the four different habitats became significantly different after 13 days under drought stress and shade, with significantly higher transpiration rates for Berhane-Kontir than for Harenna. Withholding water while exposing the plants to high irradiance revealed relatively high transpiration rates for most plants as soon as day 1 after treatment start. However, contrasting physiological behavior was already on this early stage of the experiment with significant lower transpiration rates for Berhane-Kontir than for the other habitats. Progressed soil drying lowered transpiration for all plants later on; however, Berhane-Kontir maintained higher rates at the end of the dry period in contrast to Harenna; however, Berhane-Kontir maintained higher rates at the end of the dry period than Harenna.

With regard to the treatment combinations, plants revealed different rates of stomatal conductance (g_s) in response to the specific environmental conditions (Figure 27). In the well watered treatment under shade, plants showed a very high stomatal conductance (g_s) of 0.41 to 0.71 mol m^{-2} s^{-1} (Figure 27 a). Exposing seedlings to high light intensities led to a strong decrease in g_s but with later recovery towards the end of the experiment, without, however, reaching the high initial values found at the beginning of the experimental period (Figure 27 b). In the other treatments, seedlings reacted to increasing soil desiccation by marked stomatal closure as indicated by consistently decreasing g_s values. The combination of high light intensity and soil drought led to rapid decreases in g_s only five days after imposition of stress (Figure 27 c), whereas shade-grown seedlings maintained g_s at normal values during the first week despite reduced soil moisture availability (Figure 27 d).

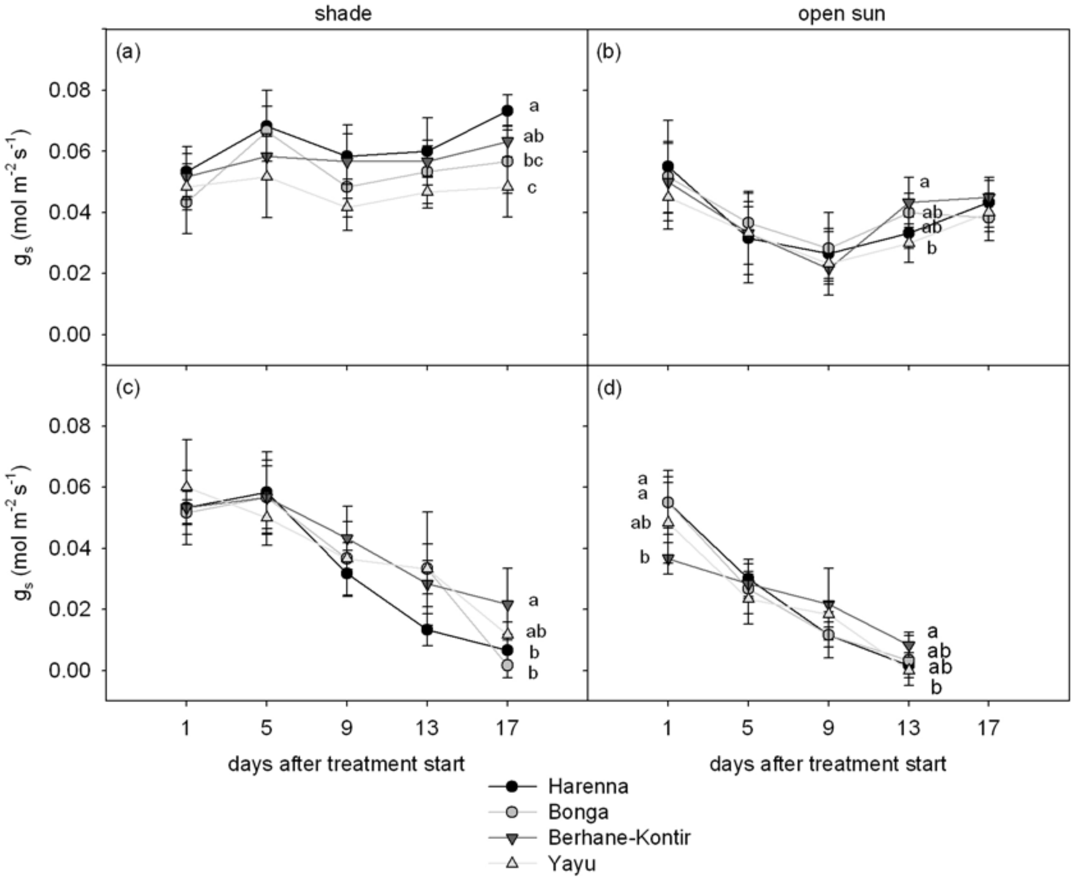

Figure 27: Mean daily stomatal conductance (g_s) of wild *C. arabica* populations from different habitats in treatment (a) shade, well watered; (b) shade, drought-stressed; (c) sun, well watered and (d) sun, drought-stressed over 17 days. Letters denote significant differences between habitats on specific days after treatment start (*P* < 0.05).

Differences in plant behavior with respect to their rates of net photosynthesis and transpiration rates also resulted in varying estimates of mean daily water-use efficiency (WUE_i; Figure 28). In the shade with irrigation treatment (Figure 28 a), WUE_i reached values of 2.0-2.5 µmol mol^{-1} and remained comparably constant over the whole experimental period. Additionally, there were no significant differences in rates of water-use efficiency among populations. Irrigation under open sun conditions (Figure 28 b) revealed that plants showed a different efficiency in water-use, with Berhane-Kontir exhibiting higher values, which is line with a tendency of lower transpiration rates (Figure 26). In shaded and drought-stressed plants (Figure 28 c), WUE_i was improved under mild soil moisture deficits in all seedlings with a remarkable increase for seedlings from Harenna; however increased water deficit under high irradiance (Figure 28 d) did not result in a remarkable increase in WUE_i. This increase results from a non-linear relationship between carbon assimilation and transpiration, with water loss being restricted to a higher degree and earlier than the inhibition of photosynthesis. At the end of the experiment, when soil moisture content reached minimum values, both drought-stressed treatments showed clear population differences with Berhane-Kontir still exhibiting water-use efficiency values comparable to the initial values, while WUE_i strongly decreased in the seedlings of other habitats.

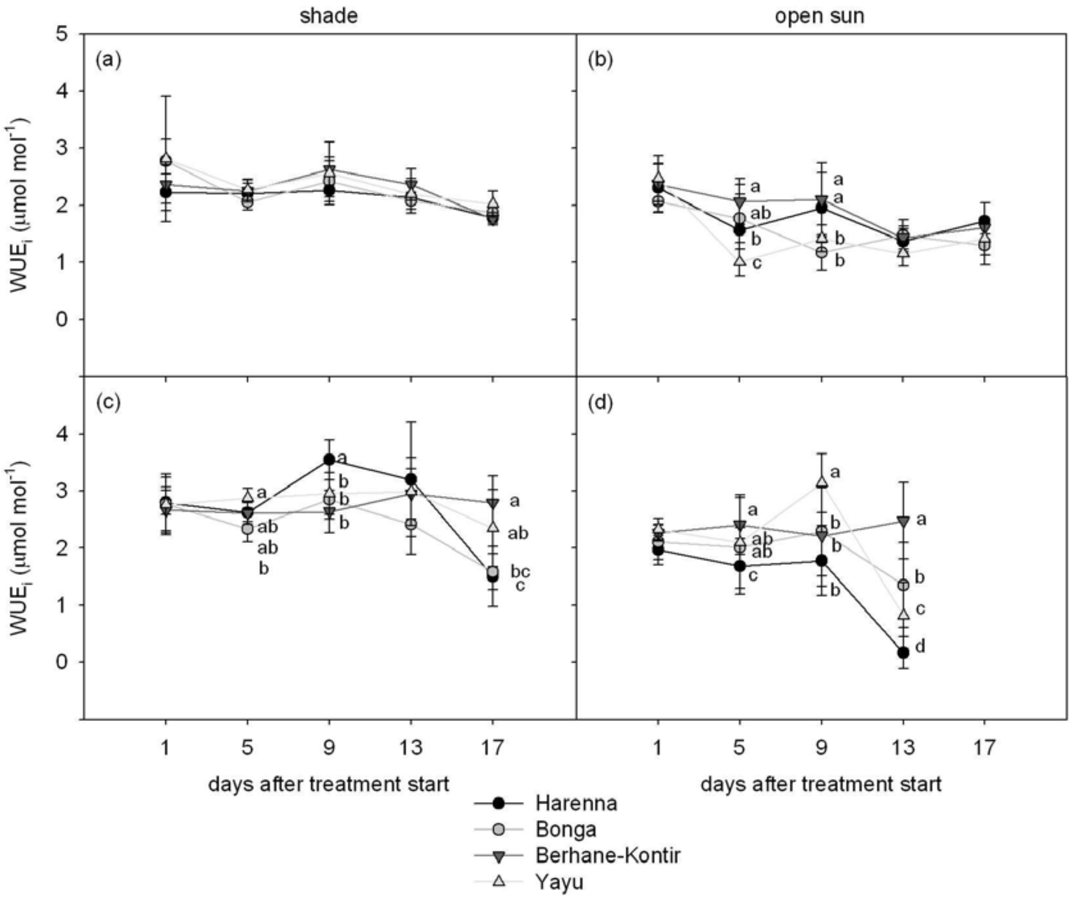

Figure 28: **Mean daily water-use efficiency (WUE_i) of wild *C. arabica* populations from contrasting habitats in treatment (a) shade, well watered; (b) shade, drought-stressed; (c) open sun, well watered and (d) open sun, drought-stressed over 17 days. Letters denote significant differences between habitats on specific days after treatment start ($P < 0.05$).**

Diurnal changes in gas exchange activity

Over the whole experimental period, the gas exchange activity of the *C. arabica* seedlings showed clear diurnal dynamics (Figure 29 - Figure 31). Results for plants from Harenna and Berhane-Kontir, the habitats with the most contrasting ecophysiological behavior over the day, revealed that wild populations experienced depression and recovery of leaf gas exchange parameters not only due to soil drought but also to daily changes in atmospheric conditions. At treatment start with a favorable soil water status (Figure 29),

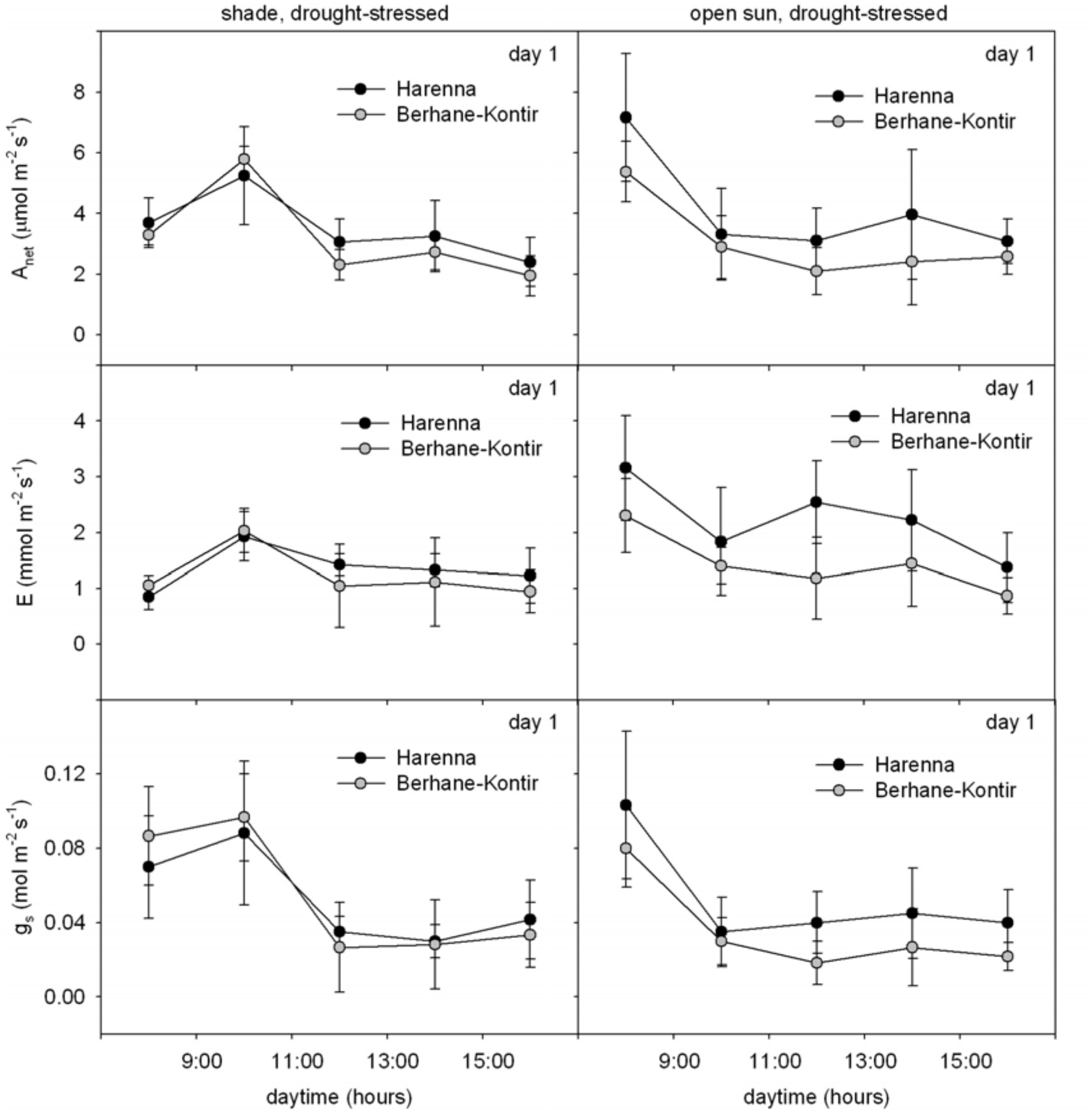

Figure 29: Diurnal patterns of net photosynthetic rate (A_{net}; µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) in wild *C. arabica* populations from the most contrasting habitats (Harenna and Berhane-Kontir) grown under shade and open sun at the beginning of a drought-stress period.

shade-grown plants exhibited a rapid early morning rise in net photosynthesis (A_{net}), transpiration (E) as well as stomatal conductance (g_s) with a peak at 10:00 am, which was followed by an equally rapid decrease in all parameters. Gas exchange parameters under open sun, however, were characterized by maximum values in the early morning (8:00 am) with a concomitant decline in net photosynthesis, transpiration and stomatal conductance. The presence of pronounced stomatal closure for Berhane-Kontir populations after midday revealed a sufficient control of transpiration when the evaporative demand of the atmosphere was high. In contrast to Berhane-Kontir, seedlings from Harenna exhibited a tendency of afternoon recovery and the afternoon transpiration rates returned approximately to morning levels. Additionally, Harenna seedlings showed a trend of higher gas exchange over the day under open sun conditions, while no habitat-specific behavior became obvious in the seedlings grown under shade.

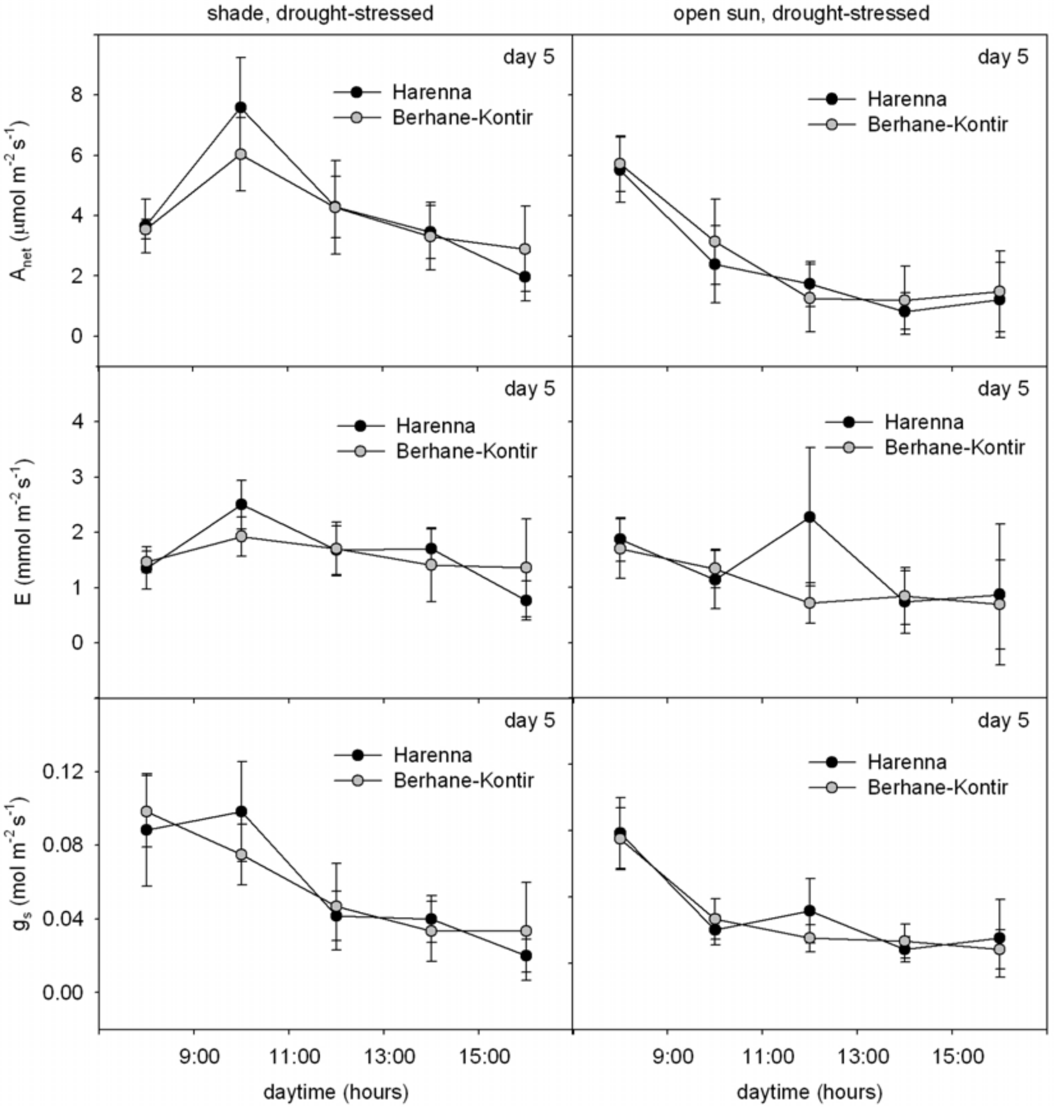

Figure 30: Diurnal patterns of net photosynthetic rate (A_{net}; µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2}s^{-1}) and stomatal conductance (g_s; mol m^{-2}s^{-1}) in wild *C. arabica* populations from two most contrasting habitats (Harenna and Berhane-Kontir) grown under shade and open sun five days after the beginning of a drought-stress period.

The results of gas exchange parameters measured 5 days after the exposition to soil drought conditions (Figure 30) revealed no population-specific differences in diurnal gas exchange activity. At the end of the experimental period (Figure 31), the diurnal fluctuations in gas exchange of the plants were depressed; however, plants from different habitats were shown to be affected to a different degree. While gas exchange rates in the Harenna populations were almost to zero with only minor activity in the early morning, seedlings from Berhane-Kontir were still able to maintain gas exchange, though at reduced rates. Under shade conditions, populations revealed different gas exchange rates; however rates became significantly different in open sun conditions.

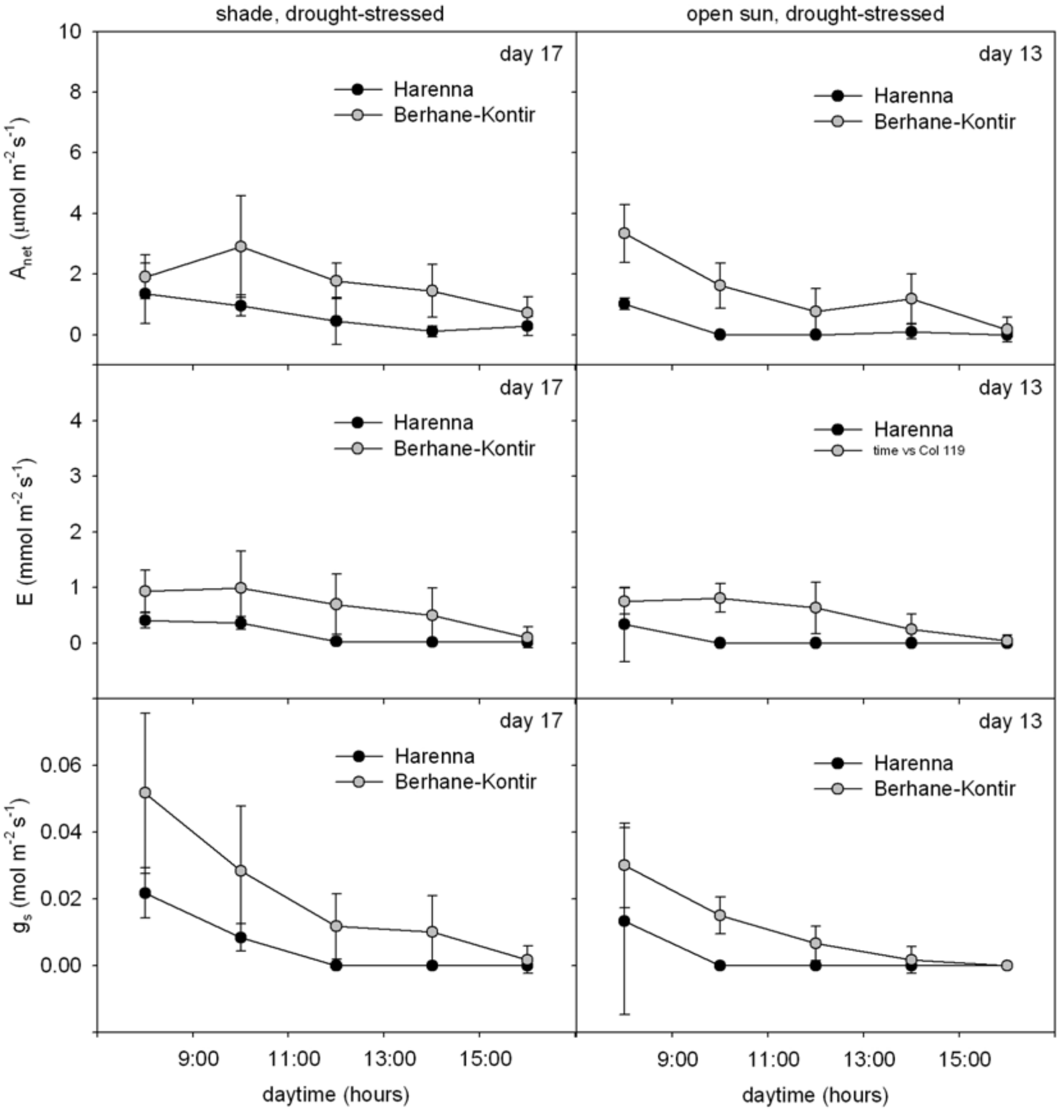

Figure 31: Diurnal patterns of net photosynthetic rate (A_{net}; µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s; mol m^{-2} s^{-1}) in wild *C. arabica* populations from two most contrasting habitats (Harenna and Berhane-Kontir) grown under shade and open sun at the end of the drought-stress period.

Dependency of gas exchange on environmental conditions and leaf water status

In order to provide a better understanding of the diurnal change in ecophysiological behavior of the plants, the dependency of gas exchange activity on changing levels of atmospheric conditions, soil moisture availability and varying leaf water status was analyzed (Figure 32 - Figure 40). Light availability measured as photosynthetic photon flux density (PPFD) influenced plants rate of net photosynthesis (A_{net}; Figure 32). Under shade conditions, maximum rates of A_{net} were reached at a PPFD value of approximately 180 µmol m^{-2} s^{-1} and an increase in light intensity above this threshold resulted in reduced rates of net photosynthesis. A much broader range of light intensity under open sun conditions, however, was followed by an increase in PPFD to values necessary for saturated net photosynthesis levels under irrigated conditions. Reduced rates of soil moisture availability at the end of the experimental period seriously affected plant photosynthesis under both light regimes with reduced maximum A_{net}.

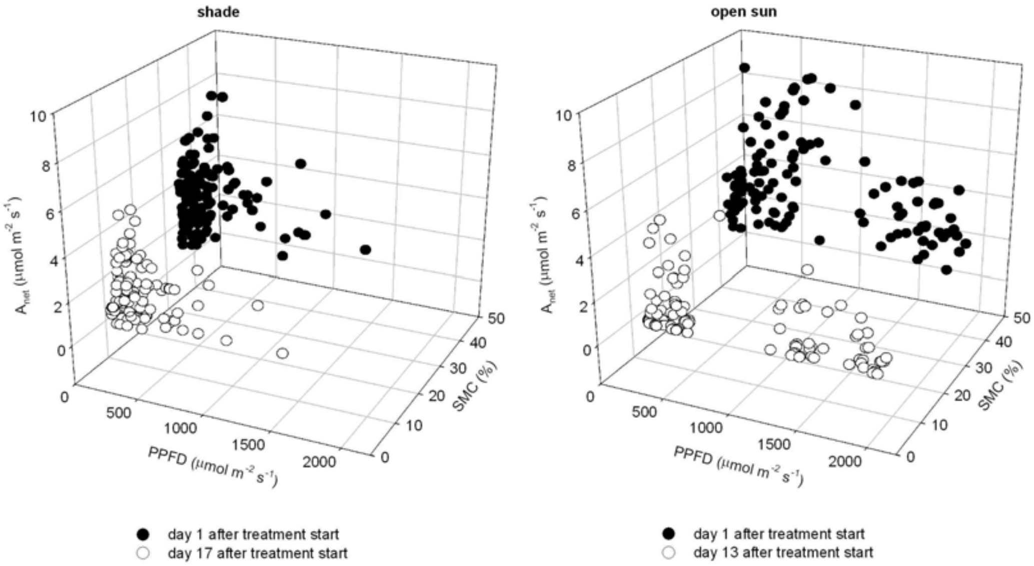

Figure 32: Influence of photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) on net photosynthetic rate (A_{net}, µmol m^{-2} s^{-1}) in wild *C. arabica* populations grown under shade and open sun over a range of soil moisture conditions (SMC).

The comparison of the light response curves of *C. arabica* populations grown under well watered and open sun conditions measured on day 1 and day 5 after treatment begin reveals that light availability had a major influence on the photosynthetic capacity of the populations under favorable soil moisture conditions (Figure 33). Immediately after the seedlings had been taken from the shade and placed in the open sun (day 1), all populations showed a rapid increase of net photosynthetic rate (A_{net}) with increasing light intensity, becoming light saturated reaching maximum values of 4-8 µmol $m^{-2} s^{-1}$ at a PPFD of about 400 µmol $m^{-2} s^{-1}$. Especially the plants from Yayu and Berhane-Kontir attained high levels of A_{max}, which shows that these populations were able to adapt to higher levels of irradiance by using a portion of the absorbed light energy to assimilate a significantly greater amount of carbon. This lowers the amount of excess energy thereby reducing the extent of photoinhibition. Except for Bonga, seedlings maintained this maximum in photosynthesis at higher light intensities with no sign of photoinhibition. However, five days after treatment start, none of the populations were able to attain values of light-saturated photosynthetic rates that were measured at the beginning of the experiment; A_{max} decreased significantly in all populations, while the PPFD necessary for saturating A_{net} was in the range found at the beginning of the experimental period.

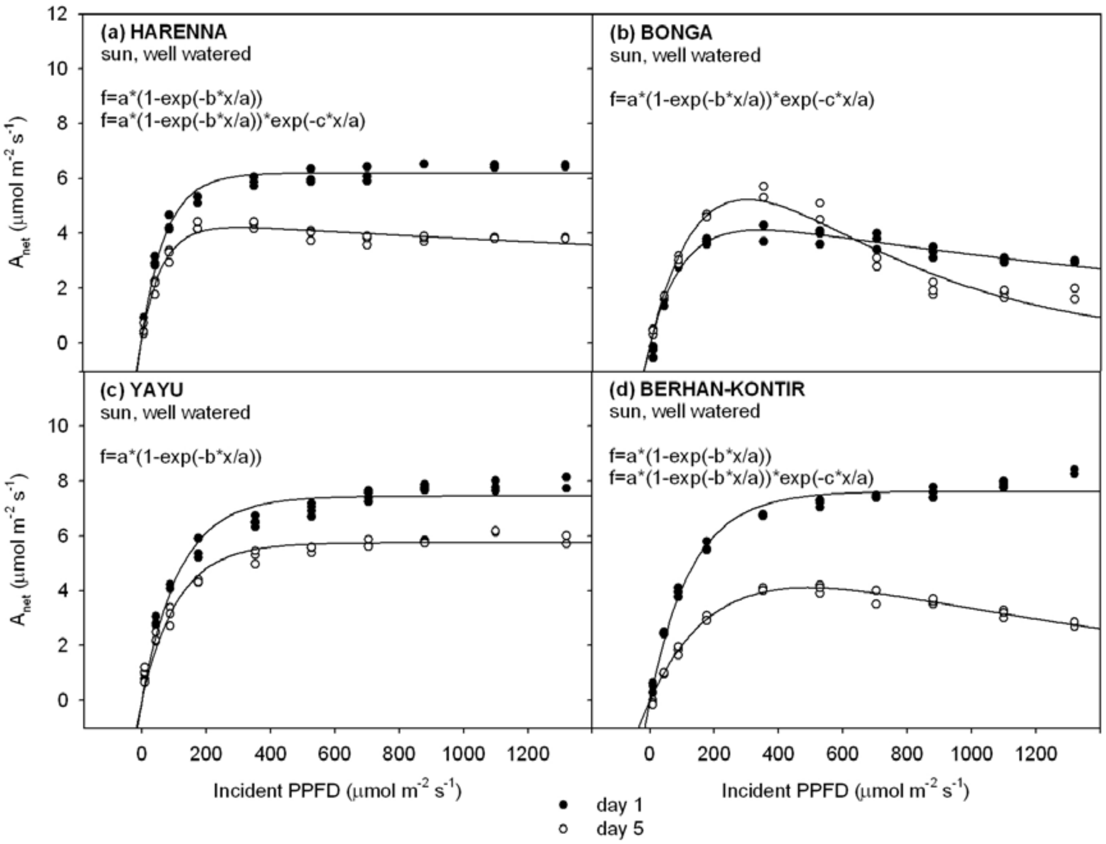

Figure 33: Response of net photosynthetic rate (A_{net}) to varying levels of light intensity (PPFD) provided by rapid light curves in wild *C. arabica* populations from different habitats measured on day 1 and day 5 after being exposed to open sun and well watered conditions.

Furthermore, when light intensity exceeded the level at which A_{max} was reached, all populations revealed photoinhibition with further increases in PPFD with exception of the seedlings from Yayu.

There was a strong curvilinear relationship between net photosynthetic rate (A_{net}) and stomatal conductance (g_s) for all populations under both light availability treatments (Figure 34), suggesting that assimilation was substantially reduced in the leaves because of stomatal limitation. Under well watered and shade conditions, net photosynthesis reached maximum values of about 5.0 µmol m^{-2} s^{-1} at g_s of 0.04 mol m^{-2} s^{-1}. Exposition to open sun altered the A_{net} versus g_s relationship with steep increases in A_{net} and an increase in stomatal conductance; A_{net} reached a maximum of 8.0 µmol m^{-2} s^{-1} at g_s of more than 0.10 mol m^{-2} s^{-1}. Reduced soil moisture availability in both light availability treatments at the end of the experimental period did not alter the shape of the regression line, but plants showed a reduced A_{net} as a consequence of depressed g_s.

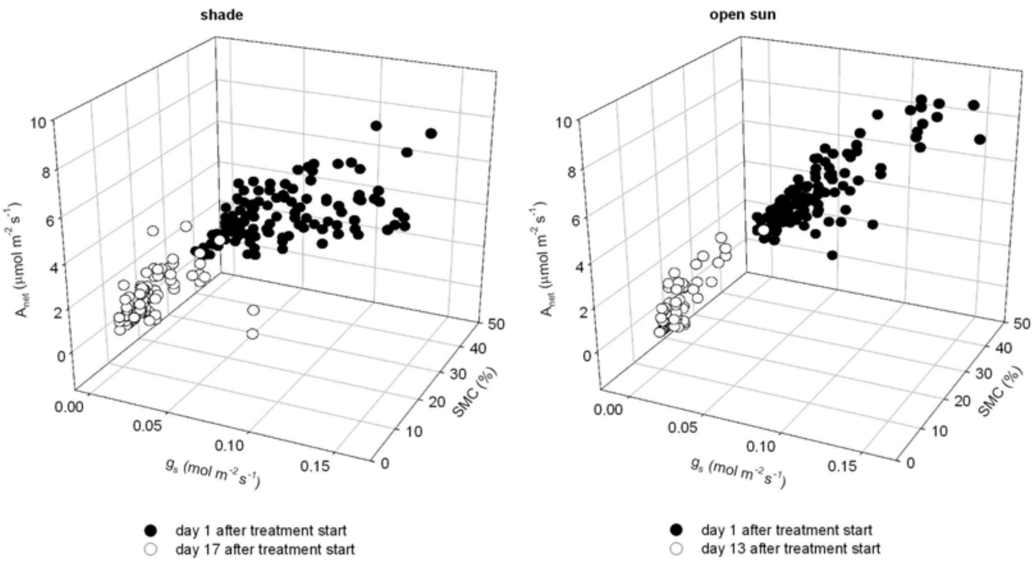

Figure 34: Influence of stomatal conductance (g_s; mol m^{-2} s^{-1}) on net photosynthetic rate (A_{net}, µmol m^{-2} s^{-1}) in wild *C. arabica* populations grown under shade and open sun over a range of soil moisture conditions (SMC).

In addition, gas exchange parameters such as stomatal conductance (g_s) and transpiration (E) were strongly influenced by varying air temperatures (Figure 38). Rates of g_s decreased with increasing air temperature in the specific light regime under well watered conditions. However, under the more moderate temperatures conditions under shade, both parameters reached a maximum at 26°C and became depressed when the temperature increased up to 31°C. The exposure to open sun and the resulting higher temperatures however, led to a strong negative relationship of T_{air} and stomatal conductance over the whole temperature range. In fact, there was a strong decrease in stomatal opening of the sun-exposed leaves due to heat stress. Despite the strong relationship of T_{air} and stomatal conductance, the dependency of E on T_{air} was rather weak with no down-regulation of water loss by transpiration in response to the influence of high air temperatures. However, when soil moisture availability at the end of the experimental period was low, maximum values of transpiration and stomatal conductance were seen to be depressed and a dependency of both parameters on atmospheric conditions as revealed under favorable soil water status could no longer be detected.

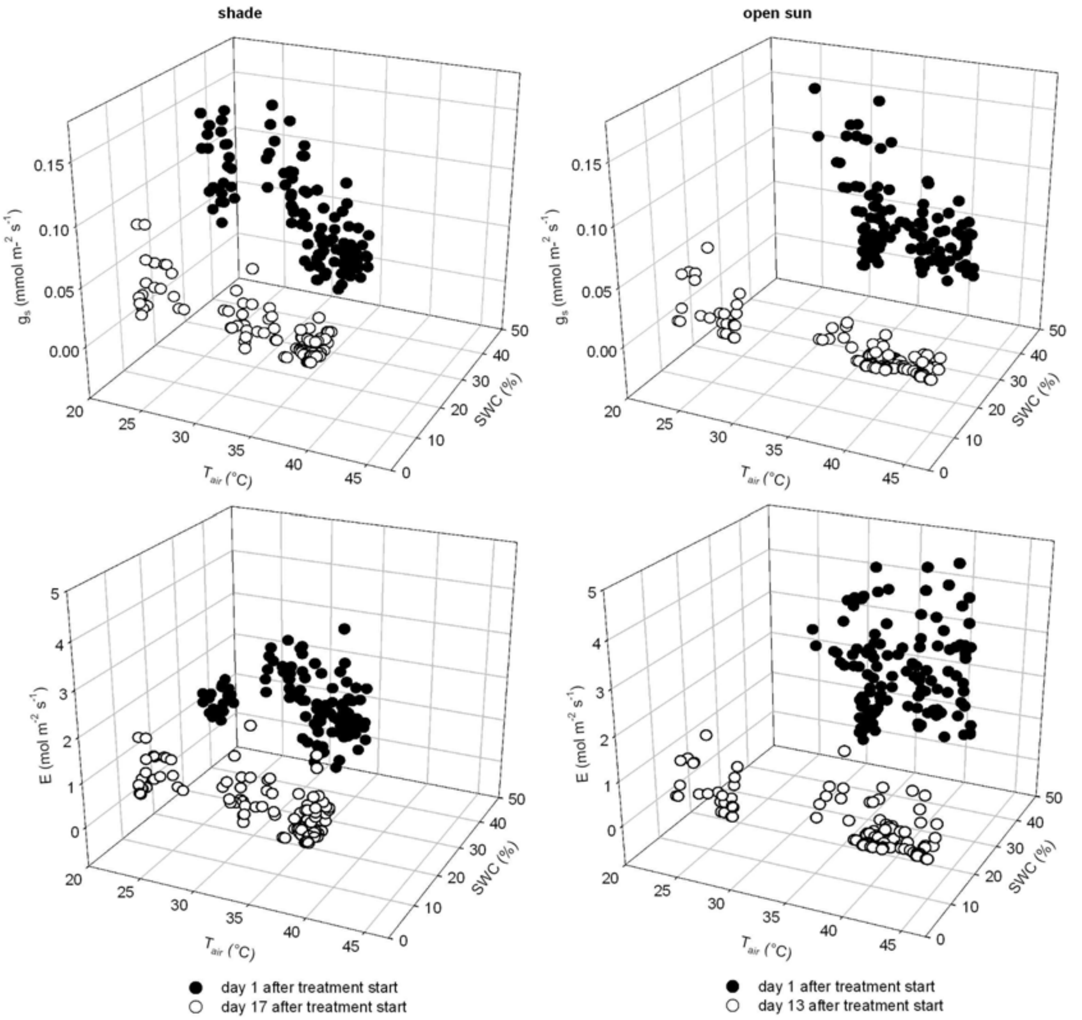

Figure 35: **Influence of air temperature (T_{air}, °C) on stomatal conductance (g_s; mol m^{-2} s^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}) in wild *C. arabica* populations grown under shade and open sun over a range of soil water conditions (SWC).**

Under both shade and open sun conditions, stomatal conductance (g_s) and transpiration (E) also decreased due to high leaf-to-air vapor pressure deficit (VPD) and the response of both parameters to changing VPD varied depending on the soil moisture content (Figure 36).

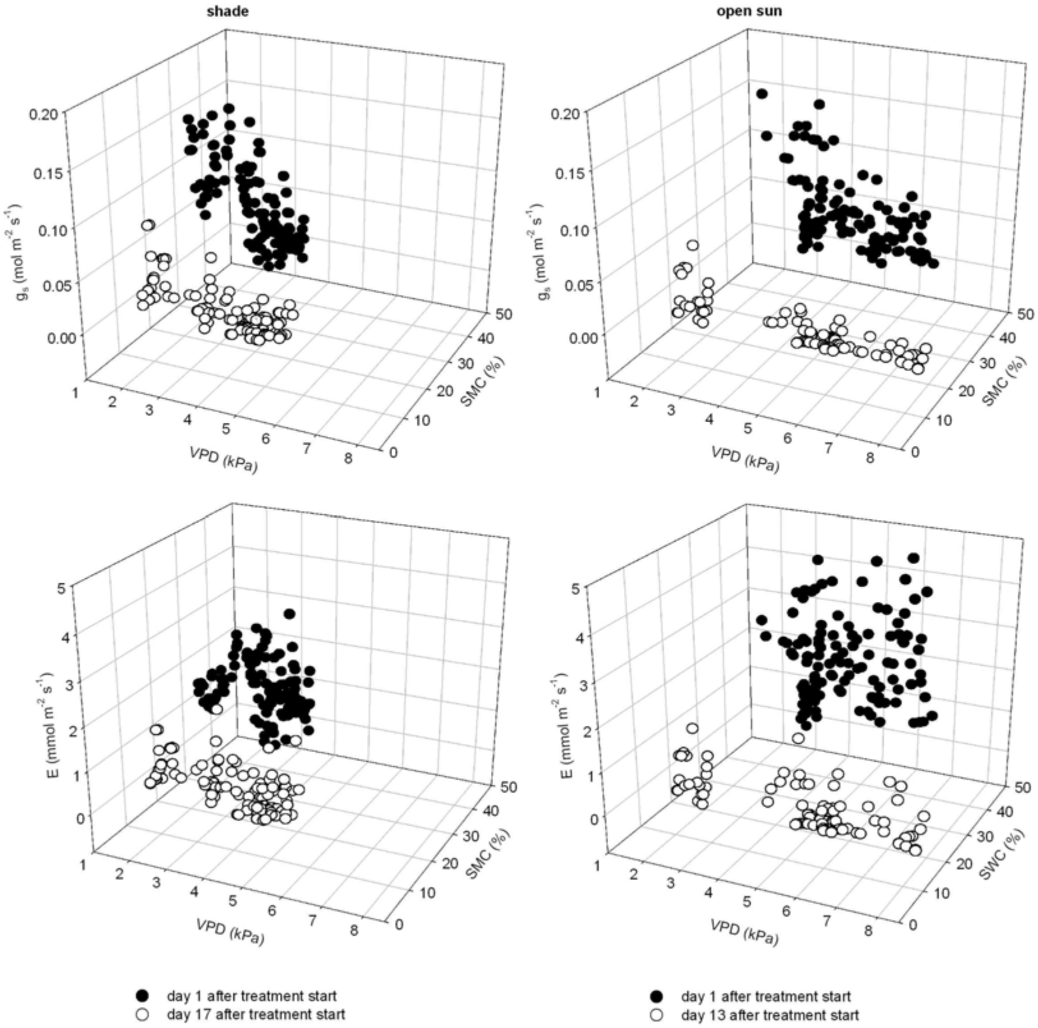

Figure 36: Influence of leaf-to-air vapor pressure deficit (VPD, kPa) on stomatal conductance (g_s; mol m^{-2} s^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}) in wild *C. arabica* populations grown under shade and open sun over a range of soil moisture conditions (SMC).

Under shade and good soil water status, there was a curvilinear relationship between VPD and gas exchange parameters, and g_s and E increased to a maximum (g_s: 0.14, E: 3) at around 2 kPa with strong decreases with further increases in VPD. In open sun, the best fit for g_s was a linear function of VPD with the highest g_s of 0.11 mol m^{-2} s^{-1} at the lowest VPD. However, the response of E to VPD was rather weak under these conditions. Hence, under shade conditions, transpiration rates (E) in plants followed the curves of stomatal conductance with decreases at high VPD and under limited soil moisture supply. Under open sun, however, a reduction in g_s under high leaf-to-air VPD was not followed by a similar decrease in transpiration rate. As soil drought stress increased, the slope of the regression lines of both parameters in response to VPD decreased to the point where there was almost no relationship; hence, progressed soil drying eliminated the response of g_s and E to the evaporative demand of the atmosphere with reductions to almost 0.00 mmol m^{-2} s^{-1} over the whole range of VPD.

The unexplained variability in transpiration in response to air temperature and vapor pressure deficit under conditions of high irradiance and sufficient moisture supply (Figure 35, Figure 36) indicates that changes in transpiration rate were not simply explainable by the evaporative demand of the atmosphere, making a more detailed approach necessary in order to understand the multi-constraint functions of E, g_s and VPD. The analyses show that there was no unique value of conductance for a given transpiration rate due to differences in VPD irrespective of the specific light regime (Figure 37). Transpiration rates showed moderate increases with increasing stomatal conductance under low VPD; high evaporative demand, however, resulted in a steep increase in E at comparable values of g_s.

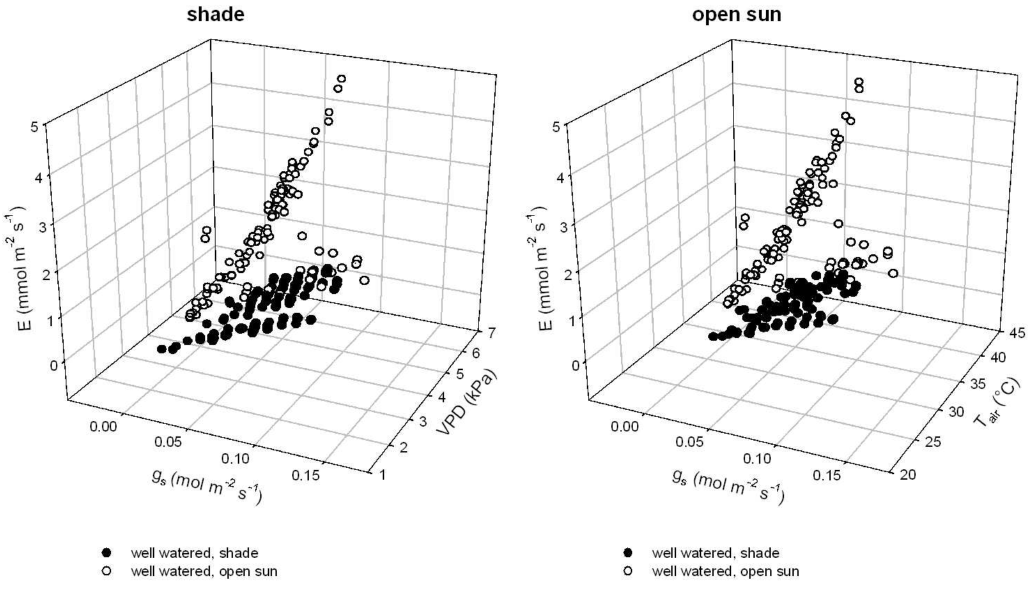

Figure 37: **Influence of stomatal conductance (g_s, mol m^{-2} s^{-1}) on transpiration rate (E, mmol m^{-2} s^{-1}) in wild *C. arabica* populations grown under shade and open sun over a range of leaf-to-air vapor pressure deficits (VPD, kPa) and air temperature (T_{air}), respectively.**

Furthermore, intraspecific variability in wild *C. arabica* was found with regard to the dependency of ecophysiological parameters on environmental conditions. Results reveal that drought stress influenced the relationship of net photosynthetic rate (A_{net}) and stomatal conductance (g_s) to leaf-to-air vapor pressure deficit (VPD) to a different degree in the selected provenances (Figure 38). For the sake of clarity, only the two habitats, Harenna and Berhane-Kontir were represented, as they showed the most significant differences. These populations differentiated in their response to the evaporative demand of the atmosphere, e.g. the Berhane-Kontir plants exhibited higher maximum values at favorable VPD and higher stomatal sensitivity to VPD, which is revealed by the steeper slope of the regression line, and hence a difference in the degree of variation in g_s with changes in VPD under sufficient soil moisture conditions (day 1). After the soil drying

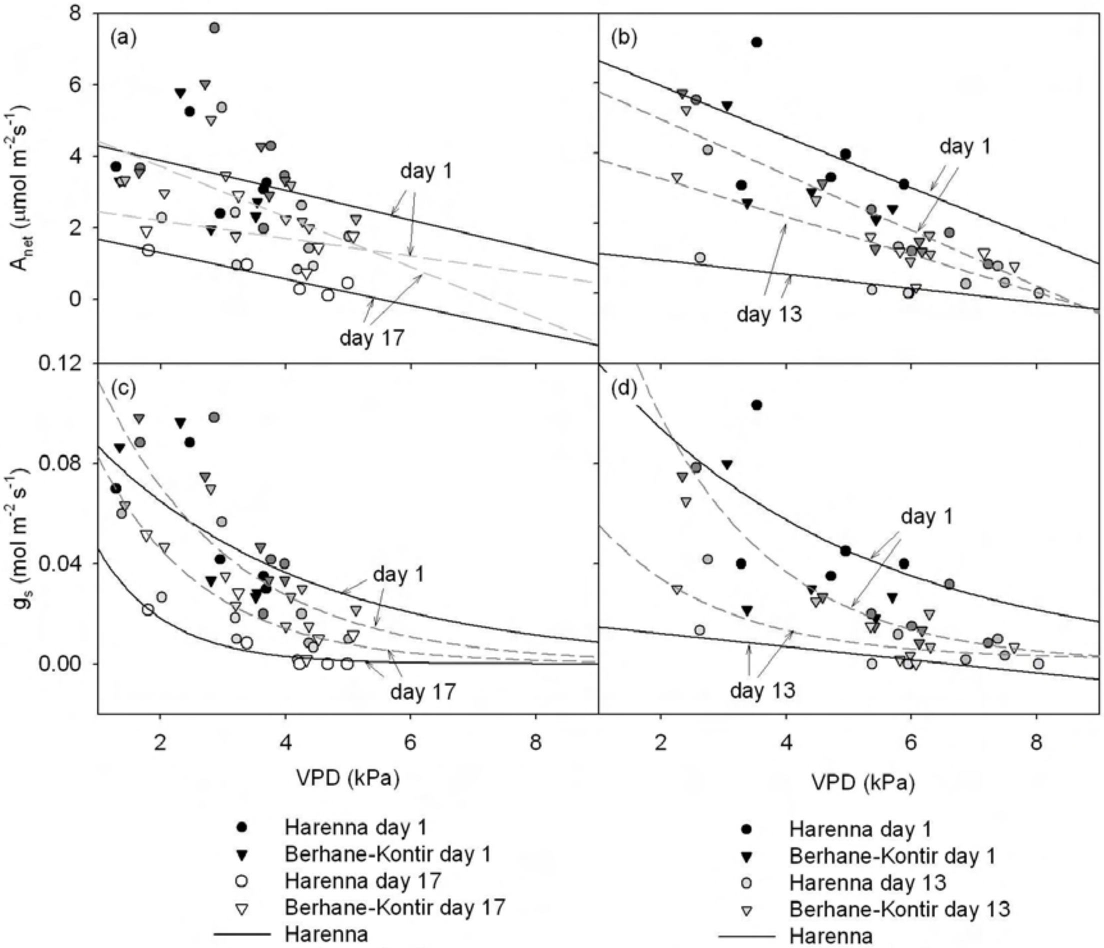

Figure 38: Influence of leaf-to-air vapor pressure deficit (VPD, kPa) on net photosynthetic rate (A_{net}), and stomatal conductance (g_s) in selected wild *C. arabica* populations grown under shade (a, c) and open sun (b, d) over a drought stress period.

period (day 17 under shade, day 13 under open sun), stomatal closure was observed at lower VPD values, which was more marked in the plants from Harenna. In addition, there was evidence that downregulation of maximum A_{net} and g_s due to drought stress was more apparent in plants from Harenna than in those from Berhane-Kontir; the latter still exhibited an ability to maintain gas exchange activity under an unfavorable soil water status.

Significant effects of soil moisture content (SMC) on transpiration rate (E) as well as stomatal conductance (g_s) were also observed (Figure 39). The initial slopes of the E/SMC or g_s/SMC relationships, which indicate how quickly both parameters declined as soil drying progressed, show that both parameters were unaffected during favorable and

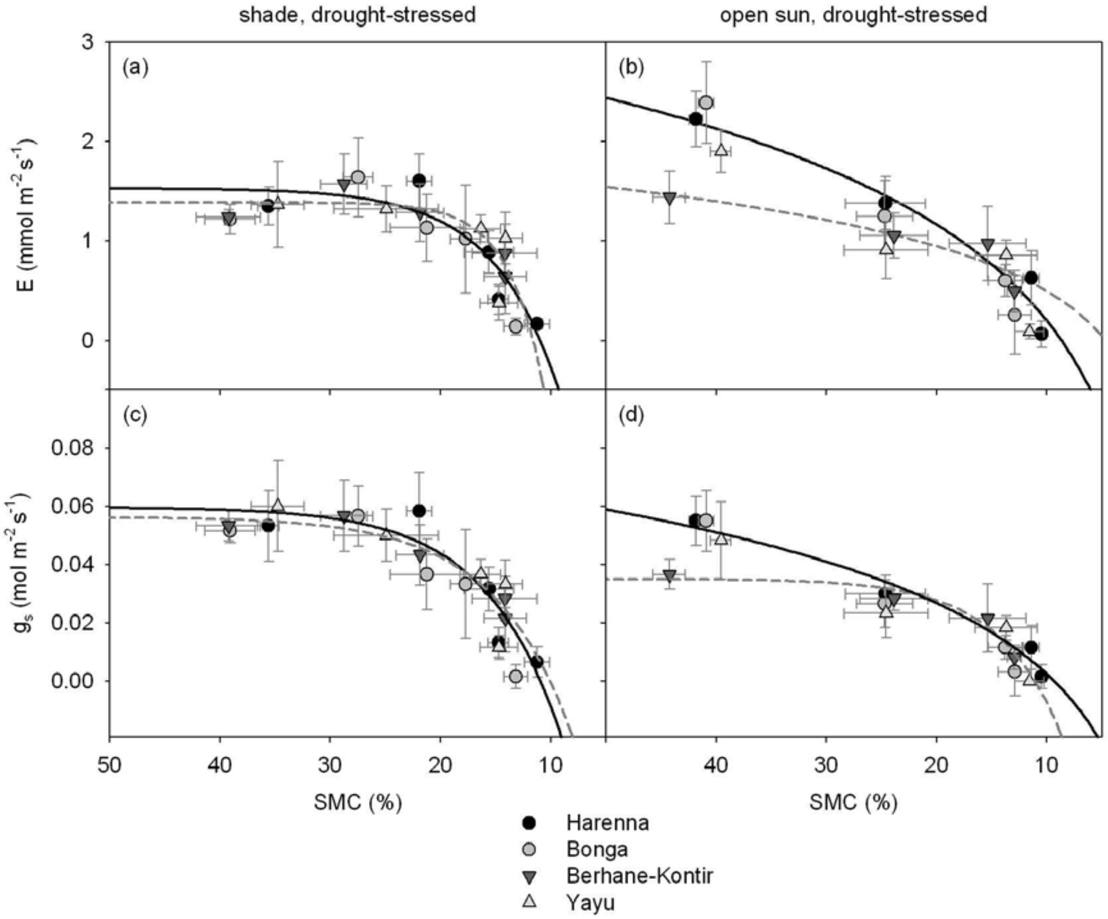

Figure 39: Influence of soil moisture content (SMC, %) on maximum daily transpiration rate (E, mmol m^{-2} s^{-1}) under shade (a) and open sun (b) and maximum daily stomatal conductance (g_s, mol m^{-2} s^{-1}) under shade (c) and open sun (d) in wild *C. arabica* populations from different habitats over a soil drying period. Regression lines represent the most different populations (– Harenna; -- Berhane-Kontir).

moderate soil moisture levels (>20 %). However, substantial inhibition in E and g_s occurred when a critical soil moisture content of around 20 % was reached and both parameters decreased sharply below this threshold value with further soil drying. Both changes in transpiration and stomatal conductance followed the pattern of decline in SMC; reduced g_s may, therefore, partly account for the decline in transpiration rates observed during the drought period. Additionally, seedlings from the different habitats differentiated in their level of gas exchange at a given value of soil moisture availability under both light regimes. For the populations exhibiting the most highly contrasting pattern, i.e. Harenna and Berhane-Kontir, it can be seen that the shade-grown Berhane-Kontir seedlings had greater reduction in gas exchange rates even under mild soil drought, while the plants from Harenna maintained stable rates of g_s and E. As a consequence, the greater decrease in gas exchange rates under moderate soil moisture conditions allowed Berhane-Kontir plants to maintain a stable leaf water status until the end of the experimental period. In contrast, high transpiration rates and stomatal conductance in Harenna when soil water contents were sufficient, rapidly and strongly influenced the gas exchange rates under conditions of severe soil drying. The differences became more obvious under open sun conditions. When the soil water status was favorable, stomatal conductance and transpiration rates for Berhane-Kontir populations were lower than those for the other habitats, which allowed these to maintain stable gas exchange rates when soil water content declined. Harenna plants, however, showed a more rapid decline in g_s, therefore being more sensitive to declining SMC.

A negative relationship was also found between predawn leaf water potential (Ψ_{pd}) and mean daily stomatal conductance (g_s) and transpiration rate (E) under both light environments (Figure 40) and both parameters appear to be an early indicator of decreased leaf water potential. For all populations, g_s and E declined with decreasing Ψ_{pd} with no apparent threshold value of Ψ_{pd} when stomata closed. In addition, differences among populations were revealed with regard to their daily maximum values of E and g_s. Regression lines reveal a strong response of transpiration rate (E) and stomatal conductance (g_s) to predawn leaf water potential (Ψ_{pd}) for all sites. Under shade, regressions lines for the most different sites, Harenna and Berhane-Kontir, revealed a linear relationship of these parameters with a gradual decline as a response to decreasing Ψ_{pd}. However, populations showed contrasting patterns in magnitude of response. Berhane-Kontir reduced daily maximum transpiration and stomatal conductance more sharply over the range of reduced Ψ_{pd} compared to the populations from the Harenna habitat (Figure 40 a, c). Also, under open sun, populations differentiated in their gas exchange rate, especially under non-stressed conditions. When Ψ_{pd} was > 0.5 MPa, Berhane-Kontir populations exhibited low rates of E and g_s in contrast to the seedlings of the other habitats, which allowed a later stomatal closure and the maintenance of a higher level of gas exchange despite further declines in Ψ_{pd}. A decrease was observed only after leaf water potential of about -1.5 MPa was reached. The other habitats, however, showed high gas exchange rates at treatment start with a steep decline in the observed parameters, which appeared to be linear (Figure 40 b, d) when Ψ_{pd} decreased.

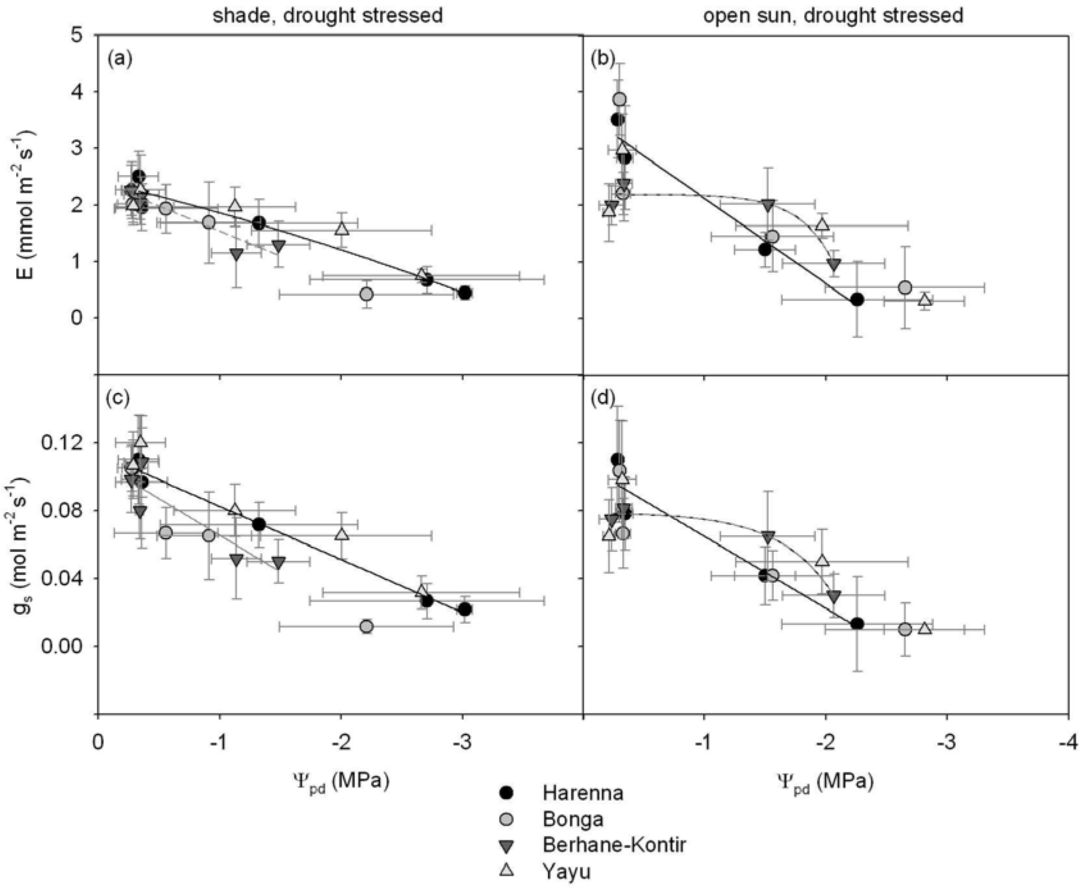

Figure 40: Influence of predawn leaf water potential (Ψ_{pd}) on maximum daily transpiration rate (E, mmol $m^{-2} s^{-1}$) and stomatal conductance (g_s, mol $m^{-2} s^{-1}$) in wild *C. arabica* populations from different habitats grown under shade (a,c) and open sun (b,d). Regression lines represent the most different populations (– Harenna; -- Berhane-Kontir).

4.3.4 Chlorophyll α fluorescence measurements

The measurements of chlorophyll *a* fluorescence show that potential and variable quantum yield of photosynthesis system II (PS II) changed according to different treatment combinations (Figure 41). While the treatment resembling natural growing conditions of wild *C. arabica* (shade, irrigated) revealed no considerable changes in these parameters over the whole experimental period, the exposition of the plants to open sun and drought stress led to a change in the chlorophyll fluorescence pattern. There were no differences among populations from the four habitats in either parameter. The maximum efficiency of energy capture of open PS II centers as analyzed by the F_v/F_m relationship (potential quantum yield) was maintained at values of around 0.7 under irrigated and shade conditions and the variable quantum yield (Φ_{PSII}) varied from 0.4 to 0.5 with only slight differences (Figure 41 a).

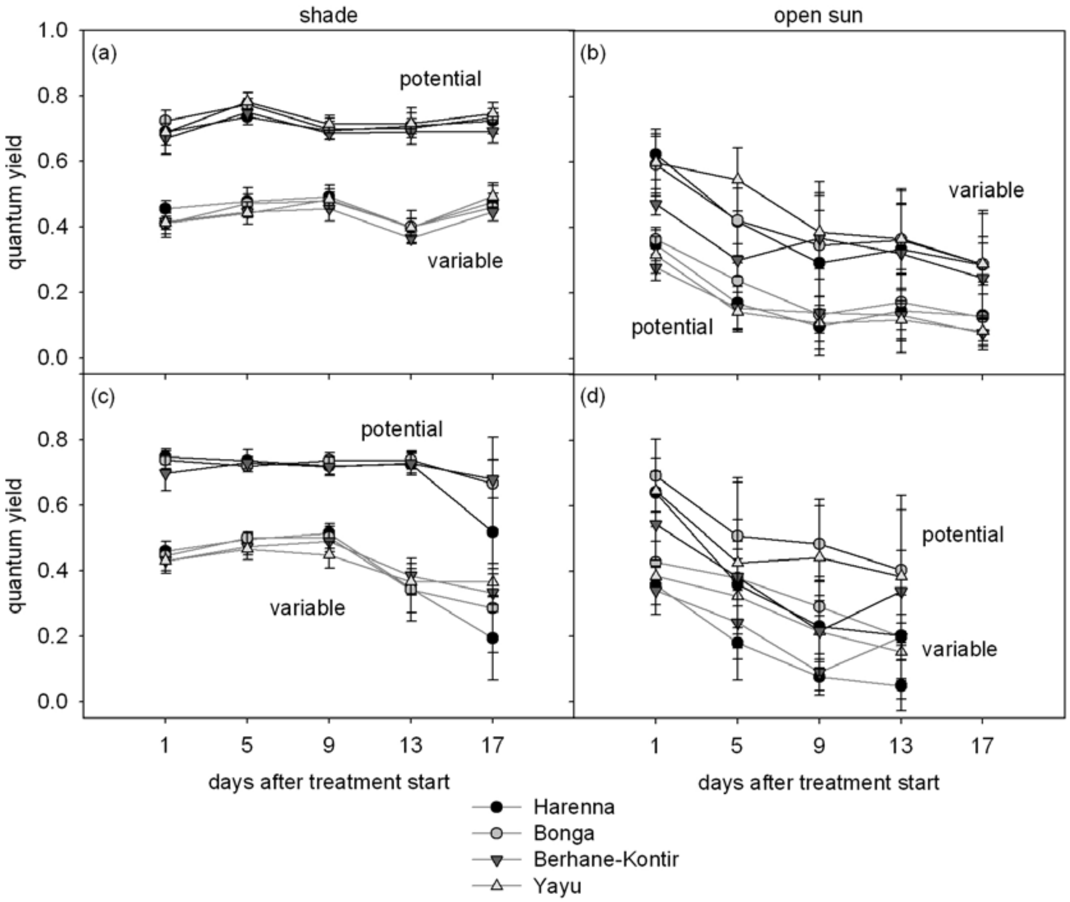

Figure 41: Mean daily potential quantum yield (F_v/F_m) and variable quantum yield (Φ_{PSII}) of wild *C. arabica* populations from different habitats in treatment (a) shade, well watered; (b) shade, drought-stressed; (c) open sun, well watered and (d) open sun, drought-stressed over 17 days period after treatment start.

Under open sun, the rapid decrease in maximum and variable quantum yield of PS II photochemistry irrespective of good water status of the soils (Figure 41 b) indicates that biochemical limitations put additional constraints on photosynthesis. However, 13 days after treatment start, both parameters as well as potential quantum yield became stable at a level of about 0.25 and 0.1, respectively, and no further decreases were detected. Under shade and non-irrigated conditions (Figure 41 c), water deficit did not alter the levels of F_v/F_m and Φ_{PSII} during the first 2 weeks after treatment start, indicating that light reactions of photosynthesis were in a fully functioning state without any signs of photoinhibition. On day 13, however, a decrease in Φ_{PSII}, which was accompanied by an unchanged F_v/F_m relationship, revealed dynamic photoinhibition. In contrast, the downregulation of potential quantum yield was also mirrored by a decrease in potential quantum yield the end of the experiment, and hence was an indicator for chronic photoinhibition. The exposure of the coffee seedlings to high light intensity and soil drought, however, was followed by strong decreases in both parameters, which reached minimum values at the end of the experimental period (Figure 41 d).

In order to simulate response to different light intensities, parameters of light-driven photosynthesis were extracted by plotting relative electron transport rates (r-ETR) versus photosynthetic photon flux densities (PPFD) at the end of the experimental period (Figure 42). While this parameter was highly influenced by the environmental conditions of the different treatment combinations, a significant habitat effect on the relationship of r-ETR and PPFD was not detected. The intrinsic capacity of the photosynthetic apparatus, defined as the maximum r-ETR at saturating PPFD, reached maximum values of 50 at light intensities of about 500 µmol photons m^{-2} s^{-1} in the well watered seedlings under shade (Figure 42 a). The imposition of stress in the other treatments, however, resulted in a significant decline in the photosynthetic capacity of the plants at a given light intensity (Figure 42 b-d). The slope of the light response curves of r-ETR is remarkably reduced under open sun conditions and severe soil drought. Furthermore, electron transport rates revealed that both incident PPFD required for light saturation of r-ETR and maximal values of r-ETR were significantly decreased, consequently a downregulation of photosynthetic capacity occurred.

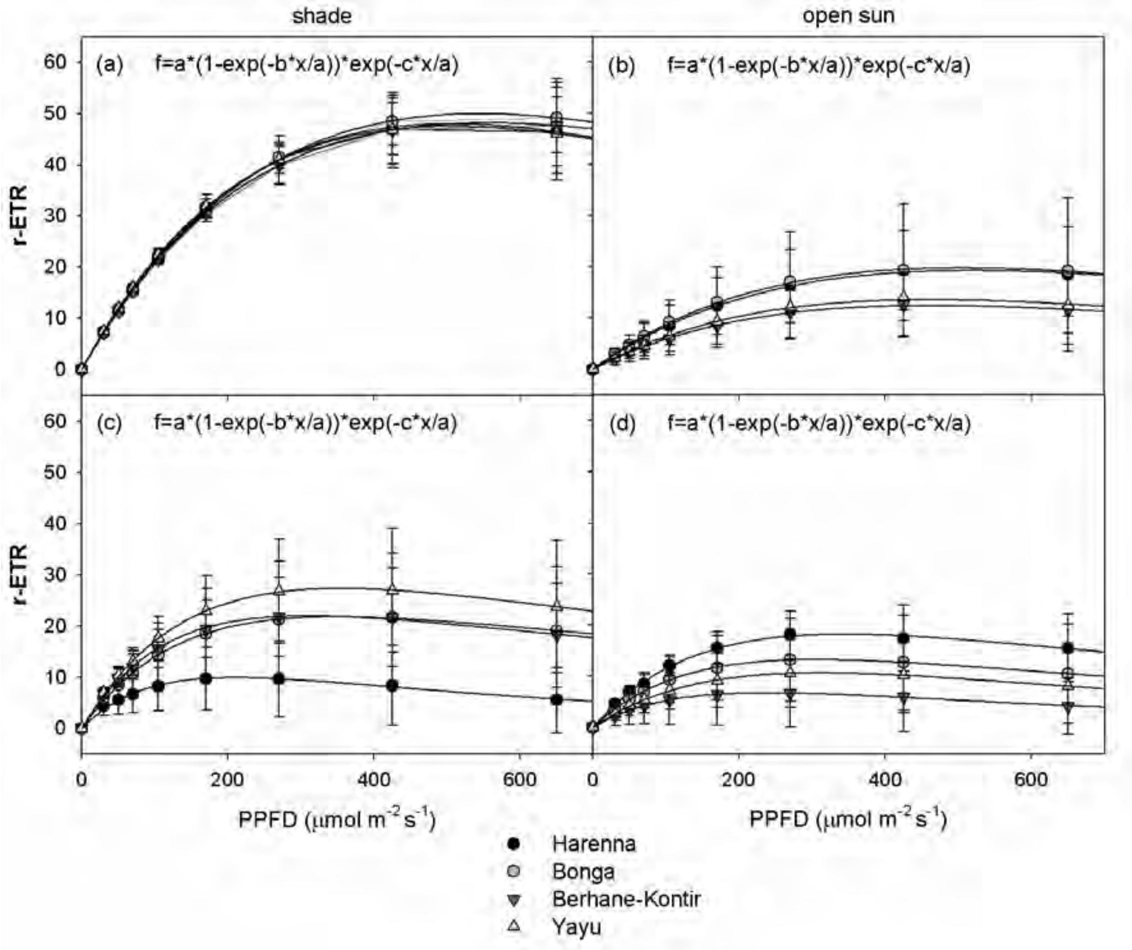

Figure 42: Influence of changing light intensities (incident PPFD, µmol m^{-2} s^{-1}) on relative electron transport rate (r-ETR) in wild *C. arabica* populations from different habitats in treatment (a) shade, well watered; (b) shade, drought-stressed; (c) open sun, well watered and (d) open sun, drought-stressed at the end of the experimental period.

4.3.5 Phenotypic plasticity and phenotypic integration

Phenotypic plasticity

In order to estimate the degree of phenotypic plasticity, the norms of reactions diagrams for each physiological parameter were plotted along the water availability treatments for the two light regimes (shade and open sun), separately for populations from contrasting habitats at each measuring date over the whole drought stress period (Figure 43 – Figure 46). While a slope of zero indicates that the environmental variable had no effect on the phenotypic expression of the trait, a non-zero reaction norm revealed phenotypic plasticity. Crossing or non-parallel reaction norms, however, are a sign of interaction.

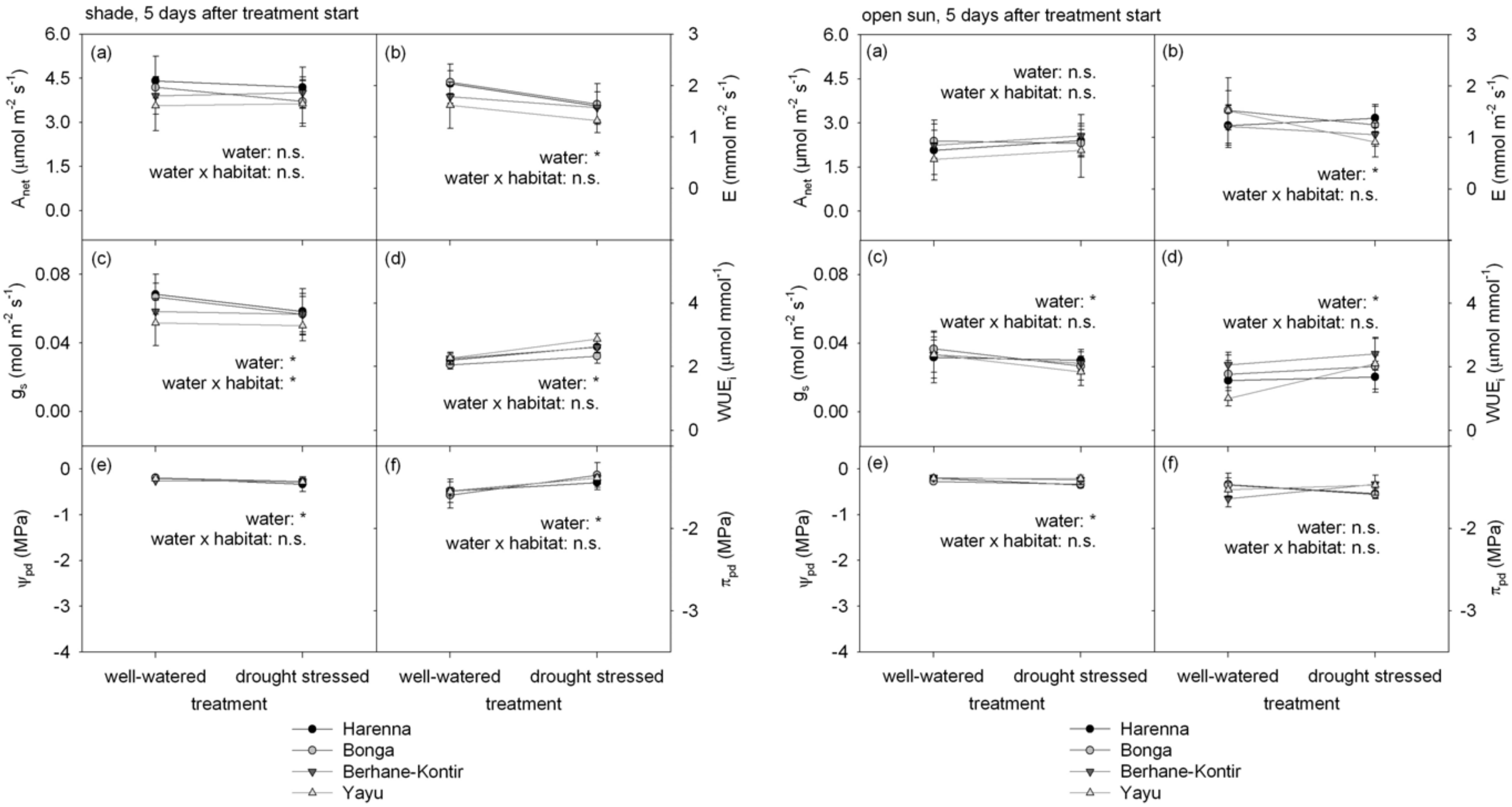

Figure 43: Reaction norms of net photosynthetic rate (A_{net}), transpiration rate (E), stomatal conductance (g_s), instantaneous water-use efficiency (WUE_i), predawn leaf water potential (Ψ_{pd}) and predawn leaf osmotic potential (π_{pd}) of wild *C. arabica* populations from different habitats in response to soil moisture conditions (well watered and drought-stressed); under shade (left panel) and open sun (right panel), 5 days after treatment start.

between habitat and environment and hence a different amount of plasticity among habitats.

While net photosynthetic rates (A_{net}) were hardly affected after five days of drought stress (Figure 43, left panel), a significant water-treatment effect for all other ecophysiological traits under shade conditions revealed that ecophysiological behavior of the seedlings was significantly influenced by withholding irrigation, which is in agreement with the expectation that water deficit induces stress. While plastic traits such as transpiration rate (E), stomatal conductance (g_s) and predawn leaf water potential (Ψ_{pd}) had higher values under non-drought-stressed conditions, instantaneous water-use efficiency (WUE_i) as well as predawn osmotic potential (Π_{pd}) of the leaves increased due to progressing drought stress. No significant habitat by water-treatment interaction was detected, indicating that the observed responses in gas exchange to drought stress were in the same direction for seedlings irrespective of their origin. However, stomatal conductance revealed a contrasting response of the populations: plants from Harenna and Bonga showed a steeper decrease in their rates of g_s due to reduced soil moisture availability than plants from Berhane-Kontir and Yayu.

A similar pattern of plasticity for ecophysiological traits of shade-grown plants was found for the seedlings grown under open sun (Figure 43, right panel). Rates of g_s, E, WUE_i changed in the expected manner due to soil drought. However, drought stress did not significantly influence net photosynthesis and leaf osmotic potential measured at predawn (Π_{pd}) at this early stage of the experimental period. Furthermore, there was no significant interaction of main factors in the ANOVA (water x habitat), i.e. differential plasticity to watering level. Hence, seedlings from the contrasting habitats were similarly affected by the respective soil drought conditions.

Nine days after treatment start, however, the watering level significantly altered the rates of all ecophysiological parameters under shade conditions (Figure 44, left panel), and gas exchange rates and leaf water status were in the same direction as found for the earlier measuring date. Also, net photosynthetic rate (A_{net}) was seen to be affected at this drought stage and was decreased by withholding irrigation. Differences among populations from the contrasting habitats were not found for A_{net}, but parameters such as transpiration rate (E) and stomatal conductance (g_s), revealed a significant habitat by water-treatment interaction, indicating that populations exhibited different levels of phenotypic plasticity. In fact, Harenna populations exhibited greater levels of plasticity in contrast to the other populations, thus showing comparably low phenotypic variation.

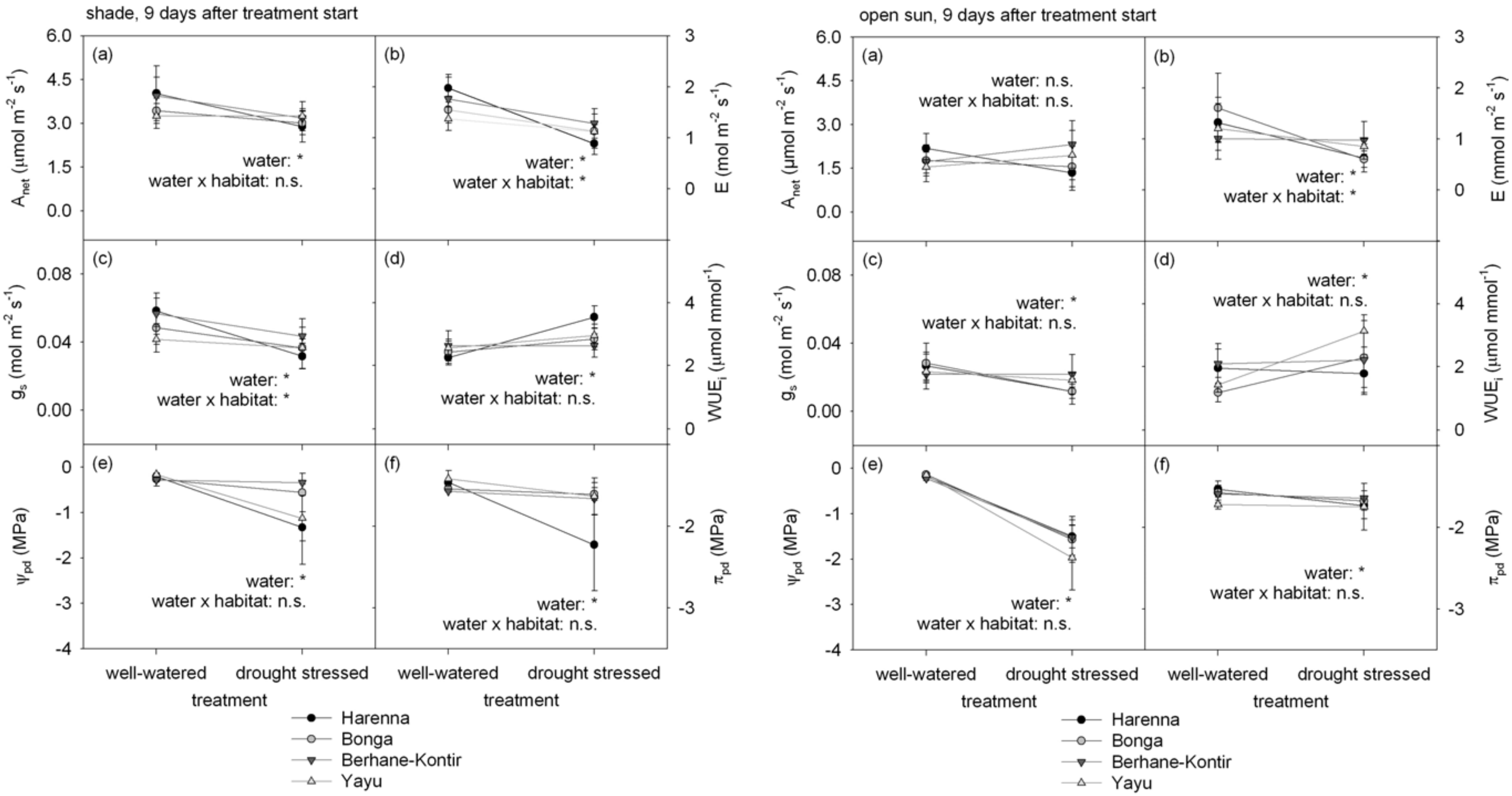

Figure 44: **Reaction norms of net photosynthetic rate (A_{net}), transpiration rate (E), stomatal conductance (g_s), instantaneous water-use efficiency (WUE$_i$), predawn leaf water potential (Ψ_{pd}) and predawn leaf osmotic potential (π_{pd}) of wild *C. arabica* populations from different habitats in response to soil moisture conditions (well watered and drought-stressed); under shade (left panel) and open sun (right panel), 9 days after treatment start.**

Plants from the Harenna populations responded to water limitation by a stronger decrease in stomatal conductance and transpiration than plants from the other habitats. As a result, Harenna plants showed a tendency of higher WUE_i under drought conditions, though the values were not significant.

Under open sun conditions (Figure 44, right panel), ecophysiological behavior showed significant effects of water regime on almost all ecophysiological parameters, indicating that the observed effect of drought as already found five days after treatment progressed in response to increasing soil water deficit. Generally, transpiration (E), stomatal conductivity (g_s) and parameters of leaf water status (Ψ_{pd}, Π_{pd}) decreased in contrast to those of the plants in the irrigation treatment. However, a non-significant effect of water for net photosynthesis (A_{net}) revealed that photosynthesis was not altered in a consistent manner by water regime; therefore, the observed increase in water-use efficiency (WUE_i) in response to drought conditions was more connected to a reduction in water loss than to differences in carbon assimilation. In addition, changes in almost all selected ecophysiological parameters were in the same direction for all populations, hence there was no overall divergence between wild *C. arabica* populations from the different habitats. The only exception was found for transpiration rates (E) where seedlings originating from Harenna and Bonga showed a larger decrease under drought stress in contrast to the Yayu and Berhane-Kontir seedlings.

The reaction norms plotted for ecophysiological parameters of shade grown plants measured 13 days after treatment start revealed a high degree of plasticity in plants behavior due to progressed soil drying as it can be seen by a significant effect of the watering level for all traits considered (Figure 45, left panel). Additionally, populations differed in their magnitude in plasticity and the most profound differences were found for plants originating from Harenna habitats in contrast to the more south-western ones. While as exchange activity and leaf water status of Harenna plants showed to be highly plastic and therefore most variable under changing environment conditions, the other populations responded rather minimally to a change in environment. Except for water-use efficiency, which showed not be significantly influenced by the watering regime, all other plant traits showed significant water regime effects after thirteen days under open sun (Figure 45, right panel). Whereas all populations showed to respond to severe water restrictions by similar patterns of plasticity in gas exchange parameters revealed by parallel reaction

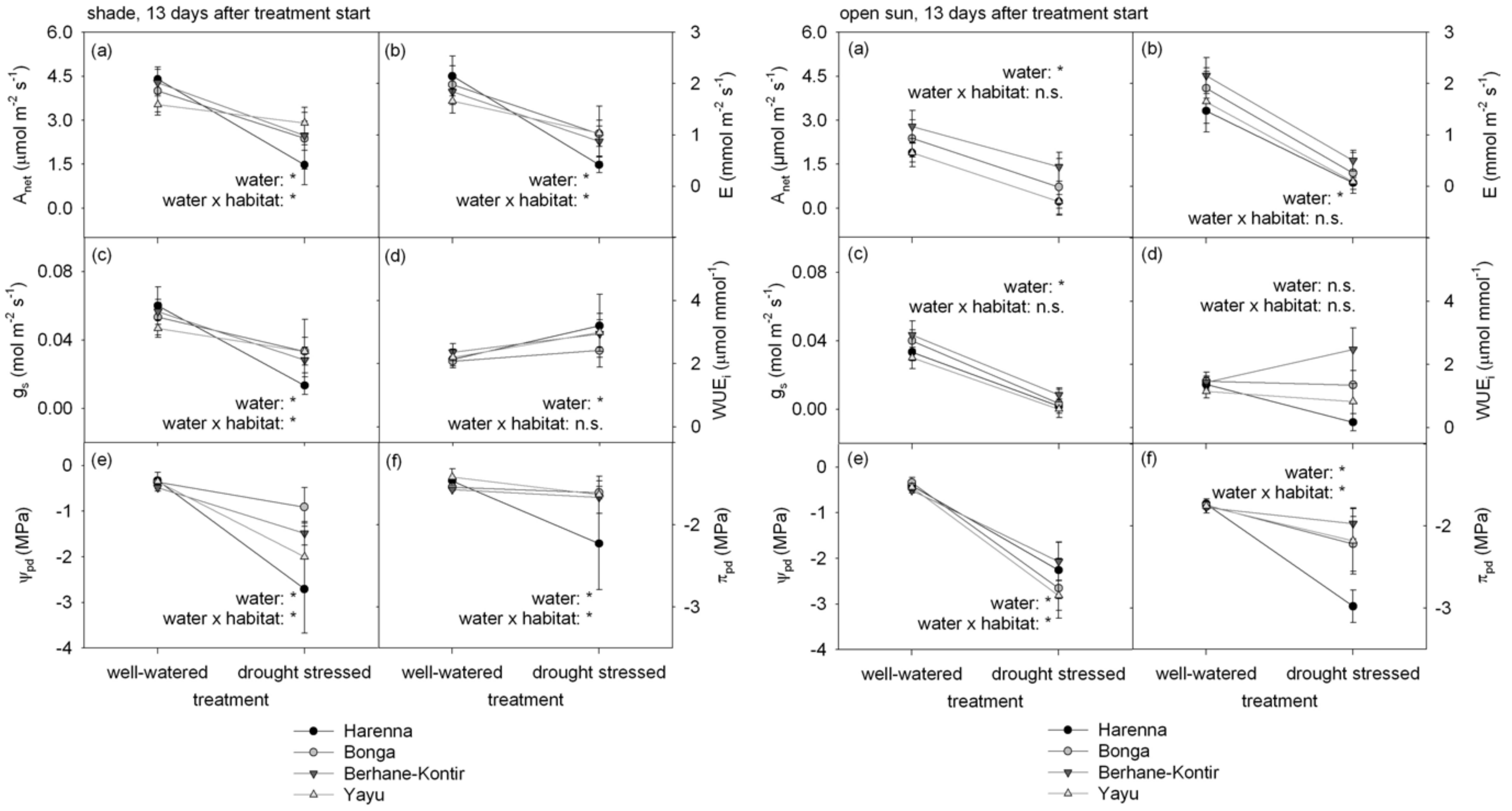

Figure 45: Reaction norms of net photosynthetic rate (A_{net}), transpiration rate (E), stomatal conductance (g_s), instantaneous water-use efficiency (WUE$_i$), predawn leaf water potential (Ψ_{pd}) and predawn leaf osmotic potential (π_{pd}) of wild *C. arabica* populations from different habitats in response to soil moisture conditions (well watered and drought-stressed); under shade (left panel) and open sun (right panel), 13 days after treatment start.

norms, tissue water relations (Ψ_{pd}, Π_{pd}) depicted differences in magnitude of response among plants from distant habitats. Plants from Harenna habitats revealed strongest decrease in their leaf osmotic as well as water potentials.

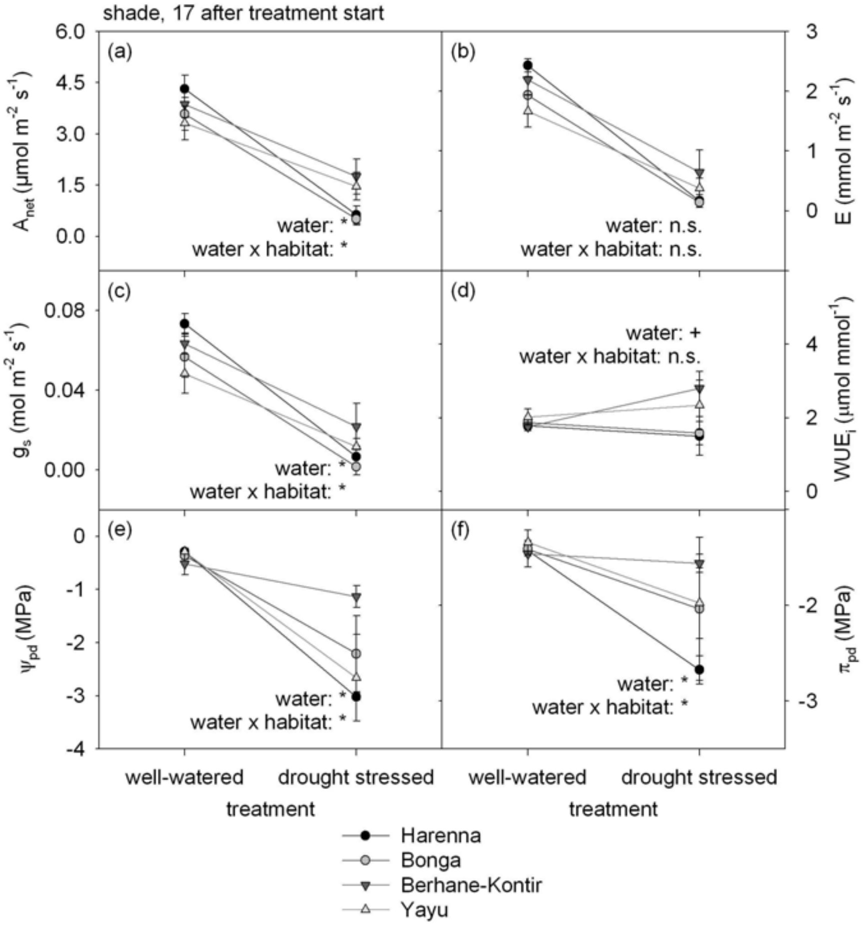

Figure 46: Reaction norms of net photosynthetic rate (A_{net}), transpiration rate (E), stomatal conductance (g_s), instantaneous water-use efficiency (WUE_i), predawn leaf water potential (Ψ_{pd}) and predawn leaf osmotic potential (π_{pd}) of wild *C. arabica* populations from different habitats in response to soil moisture conditions (well watered and drought-stressed); under shade, 17 days after treatment start.

On day 17 after the exposure of the plants to the respective treatment combinations and the last measuring date for shade-grown seedlings (Figure 46), watering level (irrigated, non-irrigated) had a significant effect on most of the ecophysiological parameters measured with the only exception of non-plastic response in transpiration (E) and values of water-use efficiency (WUE_i). In addition, for all plastic traits such as A_{net}, g_s, Ψ_{pd} and Π_{pd}, differences among seedlings from different habitats were found. Bonga and Harenna showed the highest plasticity with regard to their gas exchange activity, whereas Harenna also showed to be highly plastic in their leaf water relations (Ψ_{pd} and Π_{pd}).

Phenotypic integration

A principal component analysis considering the data set of all populations for each treatment combination was performed in order to determine whether the environment

Table 12: Principal component analysis detailing the composition of the first three eigenvectors under shade and sun conditions in the irrigated (a) and the drought-stressed treatments (b). Bold type indicates which component (within each treatment) was associated with the highest load for a given variable.

(a)	**shade, irrigated**			**open sun, irrigated**		
	PC 1	**PC 2**	**PC 3**	**PC 1**	**PC 2**	**PC 3**
Eigenvalue	3.19	2.35	1.64	2.71	2.11	21.05
% of the variation explained	31.93	23.54	16.37	27.08	21.05	16.85
π_{md}	**0.87**	-0.04	0.15	-0.02	**0.67**	-0.01
π_{pd}	**0.84**	0.00	0.10	-0.04	**0.68**	0.15
relative water content	**0.90**	0.01	0.10	0.07	**0.77**	-0.09
Ψ_{md}	**0.80**	0.12	-0.16	0.04	**0.72**	0.11
Ψ_{pd}	**0.48**	0.18	-0.25	-0.14	**0.29**	-0.21
A_{net}	0.08	**0.91**	-0.22	**0.70**	0.04	0.66
E	0.15	**0.82**	0.27	**0.90**	-0.06	-0.07
g_s	-0.03	**0.82**	0.36	**0.87**	0.02	0.42
c_i/c_a	-0.08	0.36	**0.77**	**0.78**	-0.01	-0.38
WUE_i	-0.10	-0.01	**-0.81**	-0.12	0.09	**0.92**
(b)	**shade drought-stressed**			**open sun drought-stressed**		
	PC 1	**PC 2**	**PC 3**	**PC 1**	**PC 2**	**PC 3**
Eigenvalue	4.54	2.84	1.38	4.29	3.11	1.22
% of the variation explained	45.44	28.36	13.83	42.93	31.06	12.17
Ψ_{md}	**0.95**	0.23	0.02	**0.91**	0.16	0.12
Ψ_{pd}	**0.93**	0.19	0.04	**0.93**	0.19	0.11
relative water content	**0.94**	0.18	0.02	**0.92**	0.23	0.11
π_{md}	**0.90**	0.30	-0.06	**0.90**	0.30	0.07
π_{pd}	**0.90**	0.29	-0.02	**0.81**	0.34	-0.01
A_{net}	0.27	**0.89**	0.21	0.23	**0.83**	0.43
E	0.34	**0.84**	-0.10	0.33	**0.87**	-0.01
g_s	0.23	**0.90**	-0.16	0.19	**0.92**	0.20
c_i/c_a	0.08	0.44	**-0.73**	0.24	**0.69**	-0.25
WUE_i	0.07	0.19	**0.87**	0.15	0.07	**0.94**

altered the overall structure of the correlation matrix for the specific treatment effect. Analysis was performed for populations from all habitats in order to increase statistical power. The visual inspection of the eigenvectors revealed three principal components in all treatment combinations, which are clearly differentiated into patterns of water relations, gas exchange and water-use efficiency. The first two factors, which account for the greatest variance, explained around 70 % of variation in the irrigated and 86-88 % in the drought stress treatments. While gas exchange parameters determined the first eigenvector under open sun and irrigated conditions, variation in the other treatment combinations was based more on leaf water relation parameters.

The correlation matrices of the ecophysiological parameters of the populations from the most contrasting habitats (Harenna, Berhane-Kontir) under both drought stress treatments (Table 13) show that the traits were highly interrelated in their response to

Table 13: Correlation matrix among ecophysiological characters for the two most contrasting habitats, Harenna (below diagonal) and Berhane-Kontir (above diagonal), in the (a) shade, drought-stressed and (b) open sun, drought-stressed treatment (b). Values in boldface indicate significant correlations after application of the subsequential Bonferroni procedure.

(a) shade, drought-stressed

parameter	E	A_{net}	g_s	WUE_i	RWC	Ψ_{pd}	Ψ_{md}	π_{pd}	π_{md}
E		**0.80**	**0.72**	**-0.24**	**0.28**	**0.40***	**0.42***	**0.36***	**0.40***
A_{net}	**0.92**		**0.71**	**0.24**	**0.24**	**0.36**	**0.35**	**0.34**	**0.35**
g_s	**0.83**	**0.86**		**-0.05**	**0.27**	**0.38**	**0.40**	**0.33**	**0.33**
WUE_i	0.10	**0.33**	**0.21**		-0.15	-0.04	-0.15	-0.12	**-0.17**
RWC	**0.66***	**0.60**	**0.53**	**0.27**		**0.94***	**0.95***	**0.76**	**0.83**
Ψ_{pd}	**0.71***	**0.63**	**0.59**	**0.18**	**0.66***		**0.84**	**0.55**	**0.63**
Ψ_{md}	**0.73***	**0.64**	**0.60**	**0.20**	**0.77***	**0.97**		**0.77**	**0.81**
π_{pd}	**0.64***	**0.60**	**0.53**	**0.31**	**0.98***	**0.93**	**0.93**		**0.84**
π_{md}	**0.68***	**0.62**	**0.56**	**0.27**	**0.99***	**0.95**	**0.96**	**0.98**	

(b) open sun, drought-stressed

parameter	E	A_{net}	g_s	WUE_i	RWC	Ψ_{pd}	Ψ_{md}	π_{pd}	π_{md}
E		**0.79**	**0.82**	0.04	**0.28**	**0.43***	**0.39***	**0.39***	**0.45***
A_{net}	**0.83**		**0.92**	**0.45**	**0.29**	**0.32**	**0.32**	**0.25**	**0.30**
g_s	**0.87**	**0.95**		**0.23**	**0.25**	**0.33**	**0.30**	**0.28**	**0.34**
WUE_i	**0.39**	**0.67**	**0.53**		**0.16**	-0.04	0.01	-0.13	-0.07
RWC	**0.57***	**0.49**	**0.45**	**0.56**		**0.75***	**0.90***	**0.44***	**0.63***
Ψ_{pd}	**0.64***	**0.54**	**0.52**	**0.46**	**0.73***		**0.93**	**0.84**	**0.88**
Ψ_{md}	**0.62***	**0.53**	**0.49**	**0.54**	**0.81***	**0.94**		**0.79**	**0.81**
π_{pd}	**0.62***	**0.54**	**0.49**	**0.56**	**0.99***	**0.78**	**0.92**		**0.76**
π_{md}	**0.57***	**0.50**	**0.45**	**0.56**	**0.99***	**0.72**	**0.88**	**0.98**	

drought stress. A large number of individual coefficients were statistically significant, and the magnitude of most of these coefficients was high enough to suggest biological significance. In addition, visual inspection revealed similarities in patterns of integration among the Harenna and Berhane-Kontir plants. However, plants from Harenna habitat revealed a greater magnitude and number of significant correlations than those from Berhane-Kontir, i.e. ecophysiological traits were more integrated in these populations.

5 DISCUSSION

Both *in-situ* and *ex-situ* study showed important findings regarding the ecophysiological behavior of wild *C. arabica*, which are discussed in the following. First, the general mechanisms of drought resistance in wild *C. arabica* is discussed in detail (5.1) followed by an evaluation of the dependency of plant-ecophysiological behavior on environmental parameters (5.2). In addition, the differences in drought adaptation among different wild populations are portrayed and the question is raised whether physiological characteristics were related to the environmental conditions of the natural habitats (5.3). Finally, the role of phenotypic plasticity of ecophysiological traits as an important characteristic in plants adaptation to drought is discussed in detail (5.4).

5.1 Influence of environmental conditions on the ecophysiological behavior of wild *Coffea arabica*

The findings of this study demonstrate that the ecophysiological behavior and capability to resist to drought conditions of wild *C. arabica* populations was highly influenced by the surrounding environmental conditions. Clearly, plants were exposed to variations in a number of environmental factors such as light intensity, air temperature, vapor pressure deficit of the atmosphere and soil moisture availability. Hence, especially with regard to the predictions of global climatic changes, this challenges an accurate prediction of ecophysiological behavior. Consequently, analysis of the impact of environmental variation on plants is essential in order to project ecophysiological functioning of the plants in response to changing environments.

Irradiance stress

Wild *C. arabica* grows in the shaded understory of the Afromontane rainforests, and due to the complex light conditions typically found in forest environments (Saldaña and Lusk 2003), light availability is therefore an important resource influencing plant survival and growth. Clearly, the plants are exposed to considerably changes in light intensity rather than homogeneous shade conditions (Figure 6; p. 35), which are characterized by low diffuse light alternating with sunflecks, i.e., short periods of high light intensity (Chazdon 1988). As the results of the *in-situ* study reveal, wild coffee in its natural habitat experiences a very low photosynthetic photon flux density (PPFD) of less than 200 µmol m^{-2} s^{-1}, especially during the wet season where cloud cover intensifies the effect of canopy shading (Figure 5, p. 34). However, *C. arabica* as a shade-adapted plant (Rhizopoulou and Nunes 1981) adjusts to these low irradiance levels through physiological and structural characteristics (Barber 1994). The plants exhibited an immediate increase in A_{net} in response to increasing irradiance (Figure 12 b, p. 41), thereby these dynamic responses allow an efficient utilization of sunflecks for photosynthesis (Valladares and Pearcy 1997). In addition, the plants only require low light intensity (180 µmol m^{-2} s^{-1} PPFD) for saturating net photosynthesis (A_{max}), which is in the range of earlier observations on coffee grown under shade conditions (Fahl, Carelli et al. 1994; Ramalho, Pons et al. 2000). However, light saturated rates of photosynthesis (A_{net}) of 3 µmol m^{-2} s^{-1} in the *in-situ* experiment were in agreement of other findings (DaMatta 2003) and much lower than in the shaded seedlings grown in the *ex-situ* study under common-garden conditions (Figure 32; p. 65), where A_{max} reached approximately 6 µmol m^{-2} s^{-1}. In contrast, other work (Lima, DaMatta et al. 2002; Ronchi, DaMatta et al. 2006) showed lowered rates of photosynthesis in containerized plants. However, the shade-reduced PPFD in the *ex-situ* study was slightly above that required for saturation of photosynthesis, thus there was no additional effect of photoinhibiton on ecophysiological traits detectable in these treatments. In addition, plants grown under shade exhibited a rapid and immediate increase in A_{max} (8 µmol m^{-2} s^{-1}) when transplanted to open sun conditions, suggesting that a larger percentage of absorbed light energy was used in photosynthesis (Ramalho, Pons et al. 2000), therefore having the ability to increase maximum, light-saturated photosynthetic capacity (Figure 32, p. 65). Furthermore, the level of irradiance which was needed to reach A_{max} in *C. arabica* increased when coffee was exposed to high light intensities as previously reported (Ramalho, Pons et al. 2000). However, the enhancement of A_{max} was lost over time and plants were not able to maintain this high level of photosynthetic activity (Figure 33, p. 66). Consequently, due to the general relatively large antenna complexes for maximum light capture in shade-adapted plants (Lambers, Chapin et al. 1998), the energy absorbed by these complexes was probably detrimental to the plant when it was exposed to high irradiance. Thus, a decrease in quantum efficiency of photosystem II and hence photoinhibition in high light was detectable, and a limited photosynthetic capacity at subsaturating irradiance rendered plants susceptible to photoinhibition, thus inhibiting plant carboxylation rate (Demmig-Adams and Adams 1996; Law and Crafts-Brandner 1999).

Diurnal variations in light irradiance might also influence the diurnal change of gas exchange of the plants. Under shade conditions, maximum values were found around 10 am due to an increase in PPFD, while gas exchange rates showed an early morning raise (8 am) under sun due to early favorable stages of the irradiance level. Therefore depression of A_{net} at midday found in this study could be the consequence of excess excitation energy under conditions of high incident PPFD, leading to photoinhibition of PS II photochemistry.

Heat stress

Generally, temperature is known to be another important determinant for growth and reproduction of *C. arabica* (Barros, da S.e Mota et al. 1997; Silva, DaMatta et al. 2004). As the results of the *in-situ* study show, *C. arabica* grown at its natural habitat was exposed to air temperatures (T_{air}) above the 18-21°C known as an optimal range for this species (Alègre 1959). Consequently, the negative effect due of low temperatures inducing chilling or cold stress as reported elsewhere (DaMatta, Maestri et al. 1997; Ramalho, Quartin et al. 2003) was not detected in this study. The higher temperatures resulted in a depression of ecophysiological plant performance. In particular, net photosynthesis (A_{net}) and stomatal conductance (g_s) were highest under the lowest T_{air} values and became markedly depressed with further increases in T_{air}. In the *in-situ* study, where diurnal courses of T_{air} could reach a maximum of 45°C, shaded coffee plants attained the highest A_{net} at very low temperature levels, with an optimum T_{leaf} for A_{net} of around 22°C, which is not particularly high when compared with temperature ranges experienced by open sun grown plants. However, the effect of T_{air} was of similar magnitude as that observed in earlier work (Kumar and Tieszen 1980), where coffee photosynthesis was particularly sensitive to temperatures above 20-25°C. In contrast, a tolerance to elevated temperatures together with the ability to increase A_{net} up to T_{air} of 30°C when temperatures rose gradually and a sufficiently long acclimation time was considered (DaMatta, Loos et al. 2001; DaMatta 2004) was not observed in this study. Due to the tightly coupled relationship between A_{net} and g_s (Figure 34, p. 67), the strong depression in A_{net} due to high temperature stress was mainly associated with stomatal closure at high T_{air}, especially under open sun conditions. However, a negative influence due to mesophyll impairments, which are known to progressively increase at temperatures above 26 °C, could also be assumed (Kumar and Tieszen 1980), which agrees with the findings of reduced r-ETR under open sun conditions (Figure 42, p. 77). Consequently, the heat stress was presumably provoked by a combination of both stomatal limitations and the process of disruption of the photosynthetic apparatus.

Atmospheric water deficit

In both experiments, the evaporative demand of the atmosphere measured as the leaf-to-air vapor pressure deficit (VPD) was seen to be another factor with a strong influence on the ecophysiology of *C. arabica*. Above all, stomatal conductance (g_s) was the parameter most sensitive to varying VPD (Figure 13, p. 42; Figure 36, p. 69) and low air humidity (i.e., high leaf-to-air VPD) associated with high temperature, which stimulated stomatal closure as commonly found (Schulze, Hall et al. 1982) and also described for *C. arabica* (Gutiérrez, Meinzer et al. 1994; Barros, da S.e Mota et al. 1997; Tausend, Goldstein et al. 2000). Clearly, stomatal closure was observed at critical VPD values of 4 kPa even under well watered conditions. Hence, a high evaporative demand of the atmosphere accounted for the main factor triggering midday depression of gas exchange even under irrigated conditions by a feedforward mechanism of stomatal regulation of leaf water status (Kumar and Tieszen 1980; Fanjul, Arreola-R et al. 1985; Gutiérrez, Meinzer et al. 1994; Tausend, Goldstein et al. 2000; Pinheiro, DaMatta et al. 2005). Due to the high stomatal sensitivity to atmospheric factors, there were also differences among shade and sunlit leaves regarding how long they were able to attain maximum g_s rates in the morning (Figure 29, p. 62), which is known to be closely related to coffee growth (Barros, da S.e Mota et al. 1997). The rapid increase in diurnal VPD and air temperature under open sun conditions resulted in the early and extreme depression in the diurnal range of gas exchange in sunlit leaves, while coffee grown under shade was able to maintain a relatively high gas exchange activity over a longer period in the morning. Also, transpiration rates decreased with increasing VPD, therefore reducing transpirational water loss (Maier-Maercker 1998). Thus, transpiration (E) curves can be clearly interpreted as the result of variation in stomatal behavior for the shade grown coffee plants in their natural habitat as well as in the common-garden experiment (Figure 10, p. 39; Figure 36, p. 69), with depressed E in response to stomatal closure due to high VPD, temperature and progressed soil drying. The exposure to high light intensity, however, resulted in the predicted stomatal closure in response to increasing VPD under well watered conditions but surprisingly without any drop in transpiration rate as found in other studies (Mott and Parkhurst 1991). Therefore, results suggest that in *C. arabica*, which was not expected to show patchy/heterogeneous stomatal closure as a homobaric species (DaMatta 2003), a simple feedback between g_s and VPD could not have been the reason for an increase in E. In contrast, extreme VPD levels were responsible for the uncoupling of the E-g_s-relationship, which resulted in a less efficient stomatal control on E (Figure 38, p. 71). Consequently, due to the amelioration of atmospheric conditions under shade, plants are less coupled with the atmosphere and water loss is strictly controlled through stomatal conductance. In contrast, high transpiration rates irrespective of the evaporative demand of the atmosphere under open sun conditions may induce leaf cooling, which may be a strategy used by the plants for protection against photoinhibition in order to enhance carbon fixation (Fleck, Hogan et al. 1998). Nevertheless, critical leaf water potential may develop due to the absence of stomatal limitation on water loss causing excessive xylem cavitation and failure of water transport, if soil drought develops (Tyree and Sperry 1988).

Soil water deficit

In addition to the effects of the atmosphere during drought conditions, soil water deficit caused further limitations on the ecophysiological function of the plants. In the *in-situ* study, coffee plants were exposed to changes in soil moisture content (SMC; Figure 5, p. 34) that clearly followed the seasonal variation of precipitation (Figure 4, p. 33). Specifically, during naturally occurring drought episodes, plants were exposed to SMC of less than 50 % of that found during periods of sufficient moisture supply. However, a concerted variation in different environmental factors over seasons makes it difficult to interpret the direct effects on plants separately, stressing the need to consider drought as a multidimensional stress syndrome (DaMatta 2003; Sánchez, de Andres et al. 2004). The importance of these interrelations also became obvious in the *ex-situ* experiment, where the contrasting atmospheric conditions resulted in a distinct soil drying rate under shade and open sun conditions (Figure 16; p. 45). Shading ameliorated the microclimate conditions by reducing water loss mainly due a decrease in evaporation from the soil and the leaf surface (Beer, Muschler et al. 1998); thus the sun grown plants experienced a higher water deficit imposition and lower SMC at the end of the experiment. However, there was a stronger water limitation in the seedlings in the common-garden than in the mature trees in the forests (Figure 5, p. 34), which can be attributed to the high degree of soil moisture stress experienced by the seedlings in the common-garden study.

Nevertheless, the different *ex-situ* treatments allowed the examination of the direct effect of atmospheric- and soil-related drought conditions. Interestingly, soil drought under shade did not lead to markedly different physiological responses in contrast to sun grown seedlings, hence no trade-offs between resistance to atmospheric and soil drought conditions were detectable. Under both testing environments, gas exchange was only little affected over a broad range of soil water availability conditions, so that constraints on plant ecophysiology under conditions of sufficient soil moisture were mainly due to atmospheric conditions. However, due to the protection from factors that can aggravate the impact of drought such as high air temperatures, high vapor pressure deficits and oxidative stress under shade (Holmgren 2000), water deficit conditions developed more slowly here than in open sun conditions. When minimum values of SMC were reached and plants experienced severe soil drought conditions, the soil water deficit aggravated conditions of atmospheric drought with additional negative effects on ecophysiology. In fact, the modulated soil water availability leveled the effect on atmospheric conditions on gas exchange parameters (Figure 35, p. 68; Figure 36, p. 69), e.g., the stomatal sensitivity to the evaporative demand of the atmosphere, so that plants were not able to reach their maximum gas exchange rates under favorable VPD.

In summary, especially under field conditions, these stresses occurred simultaneously, and thus this combination of stresses seriously altered plant metabolism and performance. However, the strong dependency of *C. arabica* on these factors also highlights that variability of environmental conditions is the main reason for substantial shifts in plant performance, and can therefore be regarded as crucial determinants for the physical and economical viability in coffee production systems. Especially with regard to the expectation of extended periods of high temperatures and reduced water supply due to the impacts of global climatic change scenarios (McCarthy, Canziani et al. 2001), incorporating data on the dependency of ecophysiological characteristics of the environment is therefore critical for the successful prediction of plant performance when these are exposed to environmental changes (Schulze and Mooney 1994). It allows selection of appropriate plants adapted to different environmental conditions and to draw conclusions for optimal management practices in coffee production. Therefore, its understanding may be critical for the development of new strategies and tools to enhance plant adaptation to these environmental constraints.

5.2 Determination of ecophysiological traits underlying the mechanisms of drought resistance in wild *Coffea arabica*

In order to survive and maintain productivity during dry periods, plants have to successfully adapt to reduced soil moisture availability and high light intensity, air temperature and vapor-pressure deficit (Sánchez, de Andres et al. 2004). Results of the two experiments showed that wild *C. arabica* populations adjusted their ecophysiological behavior to these conditions through different mechanistically linked combinations of physiological traits underlying their adaptation to drought stress. Hence, these findings provide important insights into the specific drought resistance mechanism in this species as well as into the possible improvement of adaptation in cultivars used in modern production systems.

Leaf water relations

Above all, plant performance strongly depended on the capability to postpone damaging effects on plant hydraulic status during drought. In the *in-situ* study, predawn leaf water potential (Ψ_{pd}) changed little over season and the minimum values during the naturally occurring dry period (Figure 7, p. 36) were above the known of values turgor loss of about -2.2 MPa previously reported for *C. arabica* (DaMatta and Ramalho 2006). Consequently, the maintenance of a stable leaf water status above this critical threshold despite severe soil drying allowed the plants to remain metabolic functionable and to be protected from xylem cavitation, which can lead to plant death (Tyree and Sperry 1988; Tausend, Goldstein et al. 2000). Also, in the common-garden study, leaf water relations were seen to be unaffected over a broad range of soil moisture conditions with stable Ψ_{pd} values of about -0.2 MPa (Figure 19, p. 50) and a relative water content (RWC) of approximately 80 % (Figure 18, p. 49), which both indicate unstressed conditions and hence good leaf water status (Nunes 1976; Tausend, Goldstein et al. 2000; DaMatta, Loos et al. 2002). However, under the severe soil drought at the end of the experiment, Ψ_{pd} felt below -2.0 MPa, and plants reached their wilting point (Figure 22, p. 53). This relatively high Ψ_{pd} of turgor loss of the plant tissues is in agreement with observations in earlier studies on drought stress for coffee (Pinheiro, DaMatta et al. 2005; Praxedes, DaMatta et al. 2006) indicative of relatively low tolerance against tissue dehydration in this species (DaMatta, Maestri et al. 1993). Consequently, the conservation of a good internal hydraulic status of plant tissues appears to be more essential than turgor maintenance per se in conferring drought resistance to the coffee plant (Vaseduva, Venkataramanan et al. 1981; Meinzer, Grantz et al. 1990; Maestri, DaMatta et al. 1995). The key role of water relations in the drought resistance strategy of *C. arabica* was also revealed by the specific drought susceptible index (*S*) (Figure 21, p. 52). As soon as tissue dehydration had developed, major constraints were placed on plant physiological activity and hence a high S in leaf water relations, which served as an indicator for a high internal water deficit, was closely related to the seedling survival rate measured in a parallel study (Kufa Obso 2006).

The leaf water relations of the coffee plants were also affected by atmospheric conditions (Slatyer 1967), revealed by the great diurnal changes in leaf water potential in the *in-situ* and the *ex-situ* experiment (Figure 9, p. 37; Figure 23, p. 54). The midday of Ψ values were relatively low, because they coincided with the time of highest evaporative demand by the atmosphere, thus integrating all environmental influences on the internal water balance of the plant (Whitlow, Bassuk et al. 1992; Sellin 1996; Mitlöhner 1998). However, the increase in Ψ at predawn revealed that the plants were able to recover the water losses over the day (Whitlow, Bassuk et al. 1992; Mitlöhner 1998; Kindeya 2003; Kindeya, Muys et al. 2005). Hence, the control and specifically the daily re-saturation capability was another important trait with regard to the ability to maintain high leaf water status during drought stress. Nevertheless, when soil were severely dry as found at the end of the *ex-situ* experiment (Figure 23, p. 54), the predawn Ψ approached the low level of midday values and the more narrow diurnal range indicated that plants lost their re-saturation opportunity under extreme drought conditions (Mitlöhner 1998).

The measurements of the osmotic potential (Π) of the coffee seedlings under common-environmental conditions show drought-induced solute changes (Figure 20, p. 51); however, this was mainly passive due to dehydration of the leaves rather than an active solute accumulation mechanism because neither turgor maintenance nor a pattern of diurnal changes, both indications of active osmoregulation, became evident (Figure 7, p. 36; Figure 24, p. 55). Hence, the coffee plants did not show osmotic adjustment as a possible strategy in drought conditions, which would allow turgor maintenance in dehydrating tissues (Ludlow 1989). However, no detectable mechanism with respect to tolerance to dehydration under extreme water deficit conditions was in agreement with the findings of low cell-wall elasticity and no pattern of osmotic adjustment in other experiments (Meinzer, Goldstein et al. 1990; DaMatta, Chaves et al. 2003). The rapid soil drying in this study may have hindered the synthesis and/or translocation of osmotic solutes, and the rate of increase in osmotic adjustment was therefore unable to match the rate of decline in leaf water potential. Consequently, the plants expressed less osmotic adjustment than field-grown plants as presented in many other common-garden studies where an active osmotic adjustment only developed in gradually developing drought stress (Turner and Jones 1980; Abrams 1988; Basnayake, Cooper et al. 1996). Therefore, a reduced rate of development of water deficit would provide a greater opportunity for osmotic adjustment and thus minimize the decrease in relative water content (RWC) per unit fall in Ψ.

Consequently, the drought resistance strategy of wild *C. arabica* could thus be clearly classified following the known categories of tolerance to and avoidance of tissue dehydration (Ludlow 1989). The coffee plants exhibited a strong desiccation avoidance strategy by means of efficient water-use and transport, which has been documented in many other studies on drought adaptation in modern coffee cultivars (Meinzer, Grantz et al. 1990; DaMatta, Maestri et al. 1993). Hence, Arabica coffee should be considered a dehydration-avoiding rather than a dehydration-tolerant species as reported elsewhere (Nunes 1976; DaMatta, Maestri et al. 1993).

Leaf gas exchange

The ability to maintain a favorable leaf water status was closely linked to gas exchange of the coffee plants, and stomatal sensitivity was one of the most efficient mechanisms protecting the plants from extensive water loss as reported elsewhere (Kanechi, Uchida et al. 1995; Pinheiro, DaMatta et al. 2005). The absence of severe dehydration in plant tissues of field grown plants during the dry season was an indication that coffee plants adjusted to the specific environmental conditions and avoided transpirational water losses through an efficient stomatal control (Figure 10, p. 39). Also, in the drought stress experiment under common-garden conditions, stomatal conductance (g_s) decreased curvelinearly with decreasing leaf water potential (Figure 40, p. 74) suggesting a strong sensitivity of g_s to plant-hydraulic properties by a feed-back mechanism (Jones 1998). While, it was not clear if g_s rates measured *in-situ* were more related to SMC or LWR, the *ex-situ* study revealed that a depression of daily rates of g_s occurred only after the soil had dried such that leaf water status of the plants had changed (Figure 39, p. 72; Figure 40, p. 74). Though an apparent threshold at which stomata closure was not apparent, stomatal conductance critically decreased at leaf potentials <-2 MPa, which coincided with soil moisture < 20 %. Hence, relatively high drops in leaf water potential around midday under high light intensities may have affected the stomatal response to VPD by a change in guard cell water potential causing stomatal closure. Specifically, g_s was associated with changes in RWC rather than with the changes in leaf turgor (Figure 22, p. 53) reported by (Meinzer, Goldstein et al. 1990). In conclusion, stomatal control relied more directly on signals specific to leaf water status and the ability to sense declining soil moisture through non-hydraulic signals (e.g., ABA) produced by the dehydrating roots, which cause stomata to close, thus restricting water loss in plants long before soil drying is extensive enough to cause dehydration of leaves (Davies and Zhang 1991; Jones 1998), was not detectable.

The net photosynthetic rates (A_{net}) of the coffee plants also declined in response to increased drought stress in both experiments (Figure 10, p. 39; Figure 25, p. 57) as observed in several other studies (Liang, Zhang et al. 1997; Cao 2000; Lawlor and Cornic 2002). The strong dependency of A_{net} on stomatal conductance (g_s) in both experiments (Figure 12, p. 41; Figure 34, p. 67) suggests that the stomatal closure can be regarded as the initial impact of drought limitation on photosynthesis (Nilsen and Orcutt 1996; Luan 2002). The depression of g_s severely limits the CO_2 concentration inside the leaf carbon uptake and the lower supply of CO_2 to Rubisco leads to a decrease in CO_2 fixation (Farquhar and Sharkey 1982) and biomass production (Chaves 1991). In addition, chlorophyll fluorescence measurements (Figure 41, p. 75) lead to the conclusion that the photosynthetic apparatus, and particularly the functioning of photosystem II (PS II), was seriously changed not only by drastic drought conditions and its effects on stomatal conductance. In fact, changes in potential (F_v/F_m) and variable quantum yield (Φ_{PSII}) of PS II photochemistry suggest that non-stomatal factors account for the down-regulation of the photosynthetic CO_2 fixation capacity in mesophyll cells (Kramer and Boyer 1995; Lima, DaMatta et al. 2002; Pinheiro, DaMatta et al. 2004). Reductions in Φ_{PSII} were observed, which is known to prevent PS II from over-excitation and photodamage (Schindler and Lichtenthaler 1996) through a regulatory decline in PS II efficiency by increased levels of energy dissipation. However, the decrease in Φ_{PSII} was accompanied by a reduction in potential PS II efficiency (F_v/F_m), thus chronic photoinhibition rather than reversible photoprotection resulted into the sustained decrease in the efficiency of photosynthetic quantum conversion (Krause and Weiss 1984; Björkman and Demmig 1987; Demmig-Adams and Adams 1992; Chaumont, Morot-Gaudry et al. 1995). However, immediately after exposure to full open sun conditions, the rapid increase in A_{net} shows the ability of a plant to acclimate to high irradiance as reported for understory plants grown in relatively high light (Ramalho, Pons et al. 2000), indicating the presence of a highly successful mechanism to hasten non-damaging energy dissipation. However, the plants were not able to maintain a high level of photosynthesis even under favorable soil moisture conditions and the down-regulation of the photochemical efficiency of PS II also resulted in lower ETR values (Figure 42, p. 77), which confirms the sustained effect on the photosynthetic apparatus resulting into dysfunctions at the chloroplast level (Bilger and Björkman 1990; Maxwell and Johnson 2000). Consequently, the damage of PS II was not only due to soil drought but also due to high light intensities. However, the parallel recovery in A_{net} and g_s after longer exposure to high light intensity, while parameters of chlorophyll fluorescence showed to be sustained suppressed, suggest that the enhancement of A_{net} was due to a decrease in stomatal limitation of photosynthesis, while a photoprotection pattern was not observed.

Water-use efficiency

Variations in net photosynthesis (A_{net}) combined with an efficient stomatal control of the transpirational water loss during the drought period in the *in-situ* study led to an improvement in instantaneous water-use efficiency (WUE_i; Figure 10, p. 39) as reported elsewhere (Jones 1992; DaMatta, Chaves et al. 2003). As indicated by a significant variation between the dry and wet season, WUE_i of all coffee plants increased as drought stress increased according to other studies (Meinzer, Saliendra et al. 1992; Ehleringer 1993). An increase in WUE_i implies that the plants were utilizing water more efficiently when soil moisture availability was reduced. However, despite adjustments in gas exchange under *ex-situ* conditions, there was no increase in daily mean WUE_i (Figure 28, p. 61) suggesting that stomatal conductance reduced proportionally both A and E rates. Thus, the plants could adapt well to seasonal water availability by adjusting WUE, especially through stomatal control, which affected the instantaneous rates of photosynthesis and transpiration, thereby optimizing carbon gain during drought. This is beneficial in drier environments, because carbon gain and growth are sustained, and soil water reserves are less rapidly depleted (Delucia and Schlesinger 1991). Under a sufficient soil moisture supply, a low WUE enabled high rates of water-use and productivity under high resource-environments (Meinzer, Grantz et al. 1990), whereas a high WUE was advantageous in dry conditions. Thus, strategies that reduce water loss lead to a higher survival rate during dry periods, whereas a more liberal use of water, associated with greater instantaneous productivity, is favorable when water is not limiting (Ehleringer

1993). In addition, the observations of seasonal adjustments in WUE in field comparisons of daily gas exchange characteristics in *C. arabica* (Figure 14, p. 44; Figure 15, p. 44) were also reflected by the analysis of $\delta^{13}C$ which integrated WUE over the specific growing season highlighted the use of $\delta^{13}C$ measures as a sensitive indicator of WUE in natural ecosystems (Farquhar and Sharkey 1982; Farquhar and Richards 1984). There was an increase in $\delta^{13}C$ values of arabica coffee during the dry season, and hence a higher WUE during periods of reduced moisture supply (Farquhar, Ehleringer et al. 1989). In combination with gas exchange it can be suggested that higher WUE resulted primarily from the maintenance of high photosynthetic capacity and down-regulation of stomatal conductance and transpiration controlling plant water status (Brugnoli, Scartazza et al. 1998).

5.3 Ecotypic differentiation in ecophysiological behavior in wild *Coffea arabica* with respect to potential water-use differences

Due to recent studies, there is widespread evidence of a high level of diversity in the wild progenitors of *C. arabica* (Montagnon and Bouharmont 1996; Aga, Bryngelsson et al. 2003) suggesting a promising value for improving resistance to environmental stresses. However, studies on the ecophysiology of *C. arabica* merely focused on modern cultivars used in commercial coffee production (DaMatta 2004; DaMatta and Ramalho 2006), which show only a narrow genetic base and hence only a limited potential in breeding, while wild populations were not included.

The wild populations of *C. arabica* grow in patches of the Ethiopian Afromontane rainforests, which strongly differ with regard to the spatial and temporal variability in environmental conditions (Figure 3, p. 18). Because dissimilar habitat conditions are expected to provoke distinct selection pressures for plants of widely distributed species (Weis and Gorman 1990; Scheiner and Callahan 1999), a different mode of natural selection is likely to promote differentiation and specialization in these populations (Stebbins 1950). Above all, the natural stands were characterized by a contrasting pattern of water availability, which is known to act as a significant selective agent in natural plant populations (Farris 1987; Dudley 1996; Dudley and Schmitt 1996; Heschel, Donohue et al. 2002). Consequently, the wild populations are expect to exhibit a contrasting pattern in water-use and the question arises if these differences can be interpreted with regard to the distinct precipitation pattern at their natural habitat.

In-situ experiment

During the naturally occurring drought conditions in the *in-situ* experiment, soil moisture availability was in a similar range for all plants, thus all coffee populations were exposed to a similar degree of drought stress (Figure 5, p. 34). Also their predawn leaf water potential (Figure 7, p. 36) was in a range above known values of turgor loss (DaMatta and Ramalho 2006), indicating that the coffee plants experienced only mild drought stress conditions. However, the coffee plants differed in their adjustment to seasonal changes in environmental conditions as shown by their gas exchange activity. Above all, the habitats with low annual amounts of rainfall such as Bonga and Harenna exhibited a better capability to maintain net photosynthesis (A_{net}) during aggravating environmental conditions, while both leaf conductance (g_s) and transpiration (E) were reduced to a similar extent in all plants irrespective of their origin at the time (Figure 10, p. 39). Hence, population differences with respect to A_{net} rates rather than to reductions in water loss resulted in distinct patterns of instantaneous water-use efficiency (WUE_i). Clearly, a tendency of higher WUE_i in plants in Harenna and Bonga provides a promising mechanism for the maintenance of productivity and hence a fitness advantage in water-deficit environments (Farquhar, Ehleringer et al. 1989). In contrast, under favorable environmental conditions, greater reliance on high levels of gas exchange activity and less efficient water-use was favorable as a component of enhanced growth and thus competitive advantage. However, gas exchange measurements generally provide only instantaneous snapshots of plant ecophysiological behavior and highly depend on specific environmental conditions, which are difficult to control (Johnson and Asay 1993; Gupta, Gupta et al. 2001). For example, gas exchange in Yayu and Berhane-Kontir was mainly influenced by air temperature and leaf-to-air vapor pressure deficit, whereas Harenna and Bonga plants depended more on light availability patterns (Table 9, p. 43). Hence, this high plant-by-environment interaction must be taken into consideration and stresses the need to interpret *in-situ* gas exchange activity with caution. Nevertheless, the finding that Harenna was more efficient in its water-use during the dry season was also reflected in the long-term WUE provided by carbon isotope discrimination measurements (Figure 14, p. 44 a), a characteristic, which is believed to show high heritability (Condon and Richards 1992). Thus, the correlation between the instantaneous and long-term WUE (Figure 15, p. 44) manifested high WUE in Harenna, which conferred its ecophysiological advantage over the other wild Arabica coffee populations. In contrast, there was a less significant correlation between precipitation amounts and WUE during the wet season (Figure 14, p. 44 a), which is in agreement with other findings (Laundré 1999). Hence, water availability did not seem to be an increasingly limiting factor in periods of sufficient water supply (Leffler and Enquist 2002), suggesting that a threshold value of seasonal precipitation exists above which additional precipitation has little impact on $\delta^{13}C$ (Leffler and Evans 1999). Interestingly, no significant differences in wood $\delta^{13}C$, known as an long-term measure of WUE (Leffler and Evans 2001), were detected (Figure 14 b, p. 44), despite the strong link to spatial climatic variability as suggested elsewhere (Switsur and Waterhouse 1988; Warren, McGrath et al. 2001). This may be to due the sampling technique; annual tree rings were not clearly visible, thus estimates of wood $\delta^{13}C$ were calculated based on pooled whole-wood cores rather than on separated tree rings. Consequently, population-specific responses to water availability were more related to their specific seasonal adjustment in water-use (foliar $\delta^{13}C$ analysis) and did not become obvious by averaging WUE over the whole life span of a plant (wood $\delta^{13}C$) because the latter did not account for high intra-annual variability of precipitation. However, populations in Berhane-Kontir were significantly different in terms of wood $\delta^{13}C$, whereas such a differentiation was not detected for the other forests. Because populations in Berhane-Kontir are likely to experience similar environmental conditions, the variation in wood $\delta^{13}C$ within this habitat should be attributed to population-specific physiological diversity rather than to microclimatical heterogeneity, which is in line with a high level of genetic diversity of the coffee plants in this habitat (Tesfaye 2006).

***Ex-situ* experiment**

The clear differences among the wild coffee populations were still valid when seeds of maternal trees examined *in-situ* were grown under identical environmental conditions in the *ex-situ* experiment. Hence, a strong genetic basis for the variability in ecophysiological behavior can be assumed, which is supported by the self-mating system of *C. arabica* (Aga, Bryngelsson et al. 2003). Also, a homogenizing effect among populations due to gene flow could be ruled out, because the studied populations were >2 km apart, so that wild *C. arabica* in diverse habitats may consist of locally specialized ecotypes rather than of adaptively similar generalist populations (Bradshaw and Hardwick 1989; Donohue and Schmitt 1999).

In the common-garden experiment simulating water limitations at an early development stage of the coffee plants, slight population differences became relevant even under optimal growth conditions. High rates of carbon assimilation and a high water-use in the Harenna plants allowed a higher level of metabolic activity and hence a rapid phenological development when soil water status was favorable (Heschel, Donohue et al. 2002; Heschel and Riginos 2005) and corresponded to their rapid germination rates and their high level of morphological productivity found in a parallel study (Kufa Obso 2006). The rapid development rates might be advantageous for these plants, because they experience drought conditions early in the growing season and hence at an earlier development stage than in the south-western habitats (Donovan and Ehleringer 1992; Heschel and Riginos 2005). Seedlings are particularly susceptible to drought due to low photosynthetic capacity and only limited carbon and nutrient reserves, thus an early root initiation and a rapid root extension would enhance the probability of early establishment of the seedlings before the onset of drought (Reid and Renquist 1997). Hence, plants reach deeper water stores sooner and are able to pursue an existence independent of their seed reserves before stress is severe enough to induce a significant effect on plants. This increases the chances of survival during drought, which was not of importance for the populations exposed to drought conditions in the late season, because a later seedling establishment is here no handicap for the growth and reproduction (Geber and Dawson 1997; McKay, Richards et al. 2003). Due to the prodigal water-use under good soil moisture conditions, Harenna plants therefore avoid extreme drought stress by completing the vegetations phase more quickly by an early and efficient biomass production, often referred to as phenological escape, which has been documented in many other plants growing in dry habitats (Fox 1990; Aronson, Kigel et al. 1992).

When plants were exposed to drought conditions in the common-garden experiment, survival and metabolic functioning was highly dependent on the maintenance of stable leaf water relations in all plants irrespective of their origin. However, the timing and severity when tissue dehydration occurred varied among populations (Figure 18, p. 49; Figure 19, p. 50) and the most contrasting behavior was revealed between the seedlings from Harenna and those from Berhane-Kontir. Specifically, the Harenna plants sustained an adequate leaf hydraulic status only over a narrow range of root-zone moisture, thus exhibiting a rather weak resistance to soil drought. By a marked decrease in leaf water relations, the critical wilting point was reached much more quickly than the plants from the south-western habitats, which resulted in a high drought susceptible index in these parameters by the end of the soil drying period (Figure 21, p. 52). In contrast, Berhane-Kontir seedlings maintained a relatively good leaf water status at similar soil moisture contents, which prevented drought-induced mortality in these seedlings better than in Harenna. Seedlings from Yayu and Bonga, however, showed an intermediate survival rate. Consequently, plants from different habitats differed in their ability to avoid tissue dehydration.

The difference in avoiding tissue dehydration under drought conditions was reflected by the gas exchange activity of the populations. Plants from Berhane-Kontir were able to maintain constant gas exchange activity in contrast to the other populations over the soil drying period (Figure 39, p. 72; (Hinckley, Lassoie et al. 1978), while there was an early reduction in gas exchange due to the early aggravation of leaf water relations in Harenna plants. However, despite their efficient control of water loss as the initial response to decreasing soil water content, leaf water potential to dropped to critical values and hence no better ability to maintain a favorable tissue water status was shown (Figure 19, p. 50). Consequently, an active water saving strategy through a fast and flexible gas exchange activity at leaf-level basis in response to soil drought was not revealed in any of the populations. Consequently, other factors might explain the differences in the degree of dehydration avoidance. For example, the initial level of gas exchange at treatment start was probably a more important determinant describing the ability of the plants to survive soil drought, and a higher water-use in Harenna under a favorable soil water status resulted consequently in the faster development of a water deficit in plant tissues. Another possible explanation is given by the contrasting ability of the plants to re-saturate their leaf tissues overnight (Figure 23, p. 54), which is in the line with the findings of the *in-situ* experiment (Figure 9, p. 37). The Berhane-Kontir populations showed the highest relaxation from midday desiccation, suggesting an increased opportunity for these plants to rehydrate overnight. Hence, their ability to avoid low predawn water potential can be seen as an important pattern in their drought adaptation and water storage mechanisms (Mitlöhner 1998; Kindeya, Muys et al. 2005). In contrast, the lowest diurnal ranges were found in populations from the Harenna habitat, and the failure to recover its predawn values led to an internal water deficit at an early stage of the experiment. Since differences in rooting pattern in pot-grown coffee plants in this early development stage could be ruled out, probably the population-specific responses to atmospheric conditions (Figure 38, p. 71) came into effect explaining the differences in resaturation capability (Figure 23, p. 54). Corresponding to the altitudinal differences among the native habitats, Berhane-Kontir plants from a low altitude experiencing higher maximum air temperatures and vapor pressure deficits exhibit a better stomatal control of water-use even under sufficient soil moisture supply. This results in a more efficient mechanism of midday depression in gas exchange when evaporative demand of the atmosphere is high and hence the preservation of a favorable plant hydraulic status. This precise pattern of feedforward stomatal control of leaf water status in the Berhane-Kontir seedlings was observed irrespective of soil water content, which supports the assumption that the success of the plants in Berhane-Kontir may have resulted from their inherently high stomatal control of water loss, which can buffer plant performance against variability in soil moisture conditions as well atmospheric drought periods by postponing internal desiccation stress (Ludlow 1989). However, under non-limiting soil moisture or brief periods of water deficit, the sensitivity of stomata is expected to be disadvantageous, because a higher midday depression in Berhane-Kontir would result in a lower CO_2 budget (Figure 29, p. 62). In contrast, the gas exchange of the Harenna plants was less responsive to atmospheric conditions (Figure 38, p. 71) and allows the maximization of crop productivity under sufficient soil moisture conditions (DaMatta, Chaves et al. 2003). Hence, population-specific sensitivity to atmospheric variations was a crucial determinant explaining differences in drought susceptibility, and the intensity and duration of the midday depression of stomatal conductance were highly important in order to predict the success of plant performance under drought. In addition, the prodigal water-use and high hydraulic conductivity found for Harenna populations under severe drought stress (Kufa Obso 2006) provides additional evidence that plants tend to live close to the limit of their hydraulic capacity (Correia and Catarino 1994). Hence, apparently small changes in one of the components of hydraulic architecture can have major implications for drought performance of the plants and predispose plants to xylem embolism, a mechanism often responsible for high seedling mortality (Williams, Davis et al. 1997).

In summary, the specific drought performance of the populations can be interpreted as two distinct strategies. Plants from drier environments were opportunistic in their water-use exhibiting high gas exchange activity when water is available (Rieger and Duemmel 1992; Zhang, Feng et al. 1997). Consequently, these plants avoided dehydration by a faster development before the onset of drought conditions. In contrast, a more defensive water-use strategy with sensitive stomatal control in the wetter habitats led to low rates of water-use, which enabled the plants to retain a good water status and to delay dehydration stress. In addition, the results revealed that the drought resistance strategies of the seedlings were more plant-inherent, hence independent of the effects of stress, than adaptive, which would have reflected the response of the plants to stress (Fussel, Bidinger et al. 1991). Hence, the ecophysiological behavior per se rather than an active acclimation pattern was important in determining plant success in the avoidance of internal plant water deficit.

Population differences in drought adaptation measured *in-situ* vs. *ex-situ*

Comparison of the *ex-situ* and the *in-situ* experiment reveals that the success of the population-specific adaptation to drought conditions varied depending on the testing environment. Plants from the drier habitat (Harenna) were well adapted to naturally occurring drought episodes under field conditions, whereas seedlings derived from these plants were highly susceptible to experimental drought in the common-garden study. In contrast, populations from stands characterized by high rainfall and short dry periods (Berhane-Kontir) exhibited a better plant performance under the testing environment. These inconsistent results have been stated for other species (Rundel and Sharifi 1993; Lauteri, Scartazza et al. 1997) and may be explained by the fact that associated ecophysiological traits in a fitness context might vary depending on specific environmental conditions (Donovan and Ehleringer 1994). Due the very fact that the Harenna plants showed a better adaptability under *in-situ* in contrast to *ex-situ* conditions reveals that the pot-grown plants do not provide a sound indication of plant performance in the field. In conclusion, the contrasting drought resistance strategies among populations, e.g. the conservative in contrast to the more offensive strategy, may be successful in one environment but not in another (Falconer 1952; Austin 1987; Van Ginkel, Calhoun et al. 1998). Thereby, such discrepancy was probably due to altered morphology and physiology under common-garden conditions (Bloor 2003), so that the results may be attributed to differences in the size of the root system between seedlings and mature trees (Clemente, Rego et al. 2005). Hence, the Harenna plants, which have been characterized by a deeper root system and a more efficient hydraulic apparatus in a separate study (Kufa Obso 2006), may have advantages under field conditions where the absence of restriction to root growth could play a substantial role in determining the degree to which these populations may succeed in a drought-stressed environment. The drought performance of the pot-grown coffee seedlings in the common-garden study could not rely on morphological adaptations such as root development, and hence the strategy of a typical water spender revealed for the mature Harenna populations failed. This led to the final conclusion that, though differences in water-use patterns among populations within a common environment imply genetic variation, this result should be interpreted cautiously however (Nilsen and Semons 1977). The genetic variation in a character is usually represented by a significant population-specific response to a specific environment, nevertheless it may be diminished or entirely masked by other limiting factors, so that genetic variability/heritability does not ensure a fitness advantage over a range of environments (Mahon 1983) and emphasizes the role of the specific field environment with regard to the expression of genetic variation of the selected characteristics (Kramer 1980).

Nevertheless, the results allowed to examine how and to what extent differences in climate and selection over the distribution range of a species result into the large variation in water-use (Sultan and Baazaz 1993; Prati and Schmid 2000). Because the among-population differentiation could be clearly interpreted in the context environmental heterogeneity of the natural habitat of *C. arabica*, thus, the contrasting behavior of the plants among the distant habitats evaluated *in-situ* and *ex-situ* provide further evidence that environmental conditions at the seed collection sites may have led to different drought-adaptation strategies among the wild *C. arabica* habitats and suggests a shift in ecological strategies at different spatial scales. The fact that plant responses could be clearly interpreted in the context of the specific environmental conditions suggests that contrasting adaptations to drought stress are related to the distinctive selection pressures due to the broad habitat range and underpins the role of the evolutionary process in the differentiation of populations in this species. Moreover, the analysis gives insights into the evolutionary processes that might have contributed to the development of climatic adaptation in plant populations.

5.4 Role of phenotypic plasticity and integration in physiological variation in response to soil drying in wild *Coffea arabica*

Phenotypic plasticity

As sessile organisms, plants experience spatially and temporally varying environmental conditions, and the capacity for plastic responses to the environment is consequently regarded as an important determinant in plant’s adaptation to spatial heterogeneity and temporal environmental change (Bradshaw 1965; Schlichting 1986; Sultan 1987).

Moreover, high phenotypic plasticity allows efficient capture of available natural resources, therefore it is not only important in for individual plant fitness, but also for the persistence of plant populations in response to climate changes and their ability to evolve in response to novel selection pressures. Consequently, expression of phenotypic plasticity has major implications for the stability and diversity of populations as well as for community and ecosystem functioning in changing environments (Schlichting and Pigliucci 1998; Sultan 2001).

In the common-garden study, various ecophysiological traits of wild *C. arabica* exhibited significant differences in their expression in response to soil drying, which can be interpreted as plastic responses to the environment (Schlichting 1986). However, the results show that clear distinguishing of plasticity of plant fitness and plasticity of its components is needed in order to fully understand the role of phenotypic plasticity in wild *C. arabica* (Lortie and Aarssen 1996). Since drought survival in a dehydration-intolerant in this species clearly depends on its ability to avoid desiccation, low plasticity in leaf water relations represents a successful drought resistance mechanism in the sense of maximizing the mean and minimizing the variation across environments. In contrast, plasticity in gas exchange parameters has the advantage of effectively avoiding tissue desiccation, and a high plasticity of these traits as important fitness components is expected to be advantageous for drought survival. Furthermore, plasticity allows a lower plant water uptake in response to soil drought, and hence to control the water resource availability by maintaining water contents in the soil over longer periods. In this study, plasticity in gas exchange in response to specific environmental conditions developed in agreement with the expected ecophysiological adaptation of minimizing water loss and avoiding tissue dehydration (Figure 43, p. 78 - Figure 46, p. 83) in agreement with previous studies (Dudley 1996; Heschel, Donohue et al. 2002; Heschel, Sultan et al. 2004). Particular phenotypic characters of gas exchange show specific directions of plasticity in response to specific environmental conditions (Sultan 1987), characterized by decreasing net photosynthesis (A_{net}), stomatal conductance (g_s), transpiration rate (E) and an increase in water-use efficiency (WUE_i) in response to contrasting soil moisture availability. While A_{net} was maintained on a constant level under mild soil drying at treatment start, E, g_s and WUE_i were the traits showing early plastic response to changing water resources. During the progressive period of soil drying, traits varied in their magnitude of plasticity and increased as soil moisture conditions became more contrasting resulting into an extremely broad range of plasticity at severe soil drought conditions.

However, the high level of phenotypic plasticity in gas exchange parameters did not result in fitness homeostasis, i.e., the ability to maintain a stable leaf water status across environments, which would have been revealed by equivalently flat reaction norms (low plasticity) of leaf water relations in the response of fitness to soil moisture gradients. Therefore, there was no fitness advantage in terms of desiccation avoidance through plastic gas exchange parameters to soil drought and hence no accurate matching of phenotype to environment. This is intriguing, because the ability to adjust the gas exchange in order to save water is thought to play an important role in plants drought resistance mechanisms. Probably, because plants may achieve stress resistance through different combinations of responses (Griffith, Kim et al. 2004), other plant factors than gas exchange activity were responsible for dehydration avoidance and stresses the need for evaluation of phenotypic plasticity at the whole-plant level as a concerted response of different traits (Ryser and Eek 2000).

In addition, the degree of phenotypic plasticity in response to soil drought was different among populations revealed by a significant habitat-by-trait interaction for nearly all ecophysiological characters (Figure 43, p. 78 - Figure 46, p. 83) as reported for other species characterized by high ecological breadth (Schlichting 1986; Linhart and Grant 1996). Hence, the observed pattern of contrasting phenotypic plasticity provides evidence of differentiation between populations of *C. arabica* growing in different habitats, which evidently select on these traits differently. The selected natural stands of wild coffee showed to differentiate with respect to the temporal variation in water availability due the seasonality_in precipitation patterns (Figure 4, p. 33) as well as to predictability of intra- and interannual water availability (Table 5, p. 30). Generally, in more variable environments such as the Harenna habitat with higher level of uncertainty of precipitation, natural selection should favor high phenotypic plasticity for characters that are adaptations to these heterogeneous soil moisture conditions, thereby buffering the plant against variable growing conditions (Bradshaw 1965; Sultan 1987). In contrast, low level of phenotypic plasticity is predicted for characters that are adaptation to environmental conditions that are likely to remain fairly constant during the lifespan of the coffee plants, as can be found in Berhane-Kontir. Accordingly, differences in degree of plasticity ranged from very low plasticity for all ecophysiological parameters in the seedlings from Berhane-Kontir to a very high level of plasticity in the Harenna seedlings, and intermediate values for the Bonga and Yayu populations. Therefore, level of phenotypic plasticity appeared to correspond to regional differences such as precipitation and drought length in accordance with theory, i.e. magnitude of phenotypic plasticity changed in line with variability of habitat conditions of the seed collection sites. Plants from Harenna consistently exhibited a flexible water-use pattern in response to soil drought and a good control in gas exchange, which indicates increased investment in water saving characteristics. However, the higher of phenotypic plasticity in gas exchange in the Harenna plants was not followed by a fitness advantage under drought stress as revealed by the reaction norms of leaf water status. In contrast, Berhane-Kontir seedlings exhibited a flat reaction norm of leaf water status and thus less dehydration stress; thereby this fitness homeostasis was maintained although no visual adjustment through rapid adjustments in gas exchange to soil moisture variation was observed. However, a flexible gas exchange activity was advantageous for Harenna under sufficient moisture supply but non in-stressful environments, whereas the consistently low level of gas exchange in Berhane-Kontir suggests that the mechanisms that improved plant performance under resource limitation may incur costs that reduce productivity in unstressful conditions (Chapin 1980, Tilmann 1988), and as a result adaptation to low-resource environments may preclude successful occupation of high-resource environments and vice versa (Grime 1979). Consequently, there was no superiority or inferiority of the different populations along the water availability gradient, which highlights the need to evaluate the plants drought resistance mechanisms under the specific environmental conditions and to clearly define the target environment, to which the coffee plant should be adapted to.

Phenotypic integration

As the results of this study show, different plant characteristics determine the mechanism of drought resistance in wild *C. arabica* and their phenological expression was highly influenced by the surrounding environmental condition as commonly found in plants (Austin 1989). Hence, an understanding of how combinations of plant characteristics influence plants performance during drought conditions is fundamental in order to reproduce a realistic whole-plant behavior (Henriksson 2001). Therefore, plastic traits should not be seen individually and stresses the need to focus on phenotypic correlations among physiological traits that are thought to be associated with integrative plant function (Geber and Dawson 1990; Reich, Ellsworth et al. 1999). However, despite the compelling evidence about local adaptation of populations, little is known about the specific combinations of traits involved in adaptation to the environment (Arntz and Delph 2001).

Accordingly, the level of phenotypic integration, i.e., the degree of correlation among phenotypic traits, can be seen as another important aspect in plant adaptation to the environment in order to describe the multidimensional phenotype of plants involved in adaptation (Schlichting 1986; Gianoli 2001).

Plastic ecophysiological traits of the wild populations of *C. arabica* were highly interrelated in this study, as shown by significant correlation among them (Table 12, p. 84). Thus, the close associations with other aspects of the phenotype suggest a high level of phenotypic integration, and hence ascertain the complexity of plant phenotype (Schlichting and Pigliucci 1998; Wagner and Schwenk 2000). Moreover, the level of correlations differed among treatment combinations, suggesting that the environment altered the pattern of phenotypic integration (Schlichting and Levin 1986). In general, drought-stressed plants showed a tighter and hence a more connected structure of trait correlation than the irrigated plants, which is in line with findings of greater phenotypic integration in more stressful environments (Schlichting 1986; Waitt and Levin 1993). Consequently, the degree of phenotypic integration increased with drought stress, suggesting a close relationship between these parameters and underlying the role of water as a major environmental factor that induces character correlations (Schlichting 1986, Pigliucci and Marlow 2001, Gianoli 2004). Moreover, this indicates that selection might operate on several ecophysiological traits in an environment-dependent manner, thereby stressful environments might exhibit a stronger selection pressure (Geber and Dawson 1990; Geber and Dawson 1997).

Comparing the seedlings from the two habitats with the most contrasting environmental conditions, Berhane-Kontir and Harenna, the sign of all the significant correlations were consistent across the drought stress treatments (Table 13, p. 85), which indicates that the physiological changes associated with plants drought response did not involve a major modification in the structure of phenotypic correlations in plants from contrasting habitats. However, seedlings showed to differentiate in the magnitude of their correlations with Harenna seedlings showing stronger correlations among ecophysiological traits. In addition, populations from distant habitats also differentiated in their relationship between environmental stress and phenotypic integration. The relationship was stronger in plants from Harenna than those from the south-western habitats suggesting that the latter could be considered less integrated in response to environmental stress. However, although there is some evidence of a positive relationship between phenotypic integration and plant fitness (Schlichting 1989), the finding of higher drought susceptibility in Harenna suggest that higher level of integration in response to environmental stress reflects rather a higher level of sensitivity to environmental influences such as soil moisture availability (Pigliucci and Schlichting 1998; Gianoli 2004). This finding deserves further exploration, nevertheless, the determination of the structure of correlations and of its environmental sensitivity supports the conclusion that phenotypic integration may have evolved as an ecological response to stress and gives a strong indication of differential intensity of selection on ecophysiological traits and the development of contrasting water-use patterns in plant populations (Lechowicz and Blais 1988; Donohue, Messiqua et al. 2000).

6 CONCLUSIONS AND RECOMMENDATIONS

The results of this study provide a comprehensive evaluation of the ecophysiological diversity of the last remaining wild *C. arabica* populations in Ethiopia and their potential in breeding for resistance to drought stress, which currently accounts for a major constraint severely affecting modern coffee production. Precisely, the findings allow predictions with regard to the adaptations of ecophysiological responses to climatic variability and an in-depth understanding of the drought resistance mechanism used by wild *C. arabica* populations. Furthermore, insights into the degree of ecophysiological diversity and the importance of natural selection in trait diversification are provided as well as into the role of phenotypic plasticity in the occupation of a broad habitat range. Consequently, the findings are highly important for the preservation of the ecophysiological diverse wild coffee populations in Ethiopia and for the development of the best conservation options. Finally, the results of this study can also be used to draw conclusions for future research.

First, the strong influence of environmental conditions on plant ecophysiology and seedling mortality under drought conditions observed in this study has important implications for understanding responses to potential climatic change. Accordingly, *C. arabica* populations are expected to be at risk due to changes in rainfall patterns accompanied by an increase in temperatures together with other elements of climate change, which will expose the plants to more frequent and severe droughts. In this regard, results highlight the use of shade as the best managing option for coffee cultivation in drought-prone environments, as it ameliorates the negative influence of drought conditions. With respect to breeding strategies, however, drought adaptation under natural field conditions cannot be replicated under common-garden conditions due to the dependence of coffee physiology on environmental influences, which makes the process of identification and selection procedures difficult and implicitly stresses the need to exactly define target environments in breeding programs as a crucial precondition.

In addition, the study reveals high ecophysiological diversity in the primary gene pool of *C. arabica*. Hence, wild populations represent a highly valuable genetic resource for modern plant breeding; thereby their incorporation in breeding programs through efficient selection will allow exploiting existing diversity for genetic improvement of modern cultivars. The study reveals that the physiological basis of drought resistance in Arabica coffee is complex and based on various ecophysiological traits that are highly interrelated. Hence, different traits have to be taken into account in breeding that aims at the development of drought-adapted cultivars. Above all, plants' ability to avoid rather than tolerate tissue dehydration seemed to the most important drought resistance mechanism, while an adaptation to longer periods of full irradiance could not be revealed in this study. Consequently, incorporating genetic material from wild populations in breeding programs provides scope for improving drought survival by selecting for more efficient mechanisms of dehydration postponement.

Additionally, broad intra-specific variation in wild *C. arabica* with regard to its ecophysiological performance was found: Populations from contrasting habitats used different strategies order to avoid tissue dehydration. The drought adaptation mechanisms ranged from a rather conservative water use and fine stomatal control of water loss to a more wasteful and more plastic water-use strategy allowing phenological escape before severe drought conditions develop. An intra-specific variation was also revealed under common-garden conditions, which demonstrates the role of inherent characteristics possessed by distant populations and suggests a genetic basis for ecophysiological diverse populations. Moreover, the specific mechanism of drought adaptation clearly corresponded to the natural habitat conditions, so that wild *C. arabica* populations in different habitats in Ethiopia appear to have accumulated high ecophysiological diversity during their evolutionary history in their natural environment. Consequently, spatial and temporal variation in habitat conditions are likely to be a major contributor to increased population divergence and hence to the high level of intra-specific variation in this species.

In summary, there is evidence of the high value of the wild coffee populations with regard to their ecophysiological diversity, which emphasizes the need to protect them from threats through habitat degradation in a highly disturbed area in order to assure that genetic resources are available for present and future needs. Hence, the specific findings of this study provide important insights from which major conclusions for the development of conservation strategies can be drawn: First of all, there is immense intra-specific ecophysiological variation in wild *C. arabica*, and populations exhibit different strategies in their adaptation to different environmental conditions, which allow them to occupy a broad range of habitats. Thus, it is necessary to focus on conservation strategies for a variety of populations within the primary gene pool of wild *C. arabica*. Clearly, plants from different habitats should be selected rather than focusing on one single population, because each could be advantageous for specific target environments at which breeding efforts are aiming. In addition, the immense role of natural selection in the diversification of ecophysiological traits stresses the need for an *in-situ* conservation approach in order to allow co-evolutionary processes the natural habitat, especially with regard to producing resistance to environmental stresses. This conservation strategy would also avoid the risk of losing valuable germplasm due to poor adaptation of selected plants to the local environment as a possible consequence of the high genotype-by-environment interaction, which could be the case when following the option of *ex-situ* field gene bank conservation. Therefore, *in-situ* conservation is an important component of the overall strategy for the long-term conservation of the coffee gene pool.

However, there were some limitations in the study, which call for further research:

The results reveal that ontogenetic effects are likely to be significant in evaluation of drought adaptation in wild *C. arabica*. While this study mainly focused on drought adaptation at an early development stage of the plants, the feasibility of scaling from seedlings from mature trees will depend on thorough evaluation of the ontogenetic effects and how they vary under different environmental conditions. Hence, studies on the sources of phenotypic variation throughout the ontogeny of this species remain essential to extend the findings of this study.

In addition, the maintenance of acceptable yield under unfavorable growing conditions is the most important requirement in drought adaptation. Thus, the role of functional ecophysiological traits associated with reproductive fitness and hence, whether or not a specific ecophysiological trait is of technological importance should be the subject of further research. To understand how the suite of characters influences fitness components of yield is, therefore, a challenge of abiotic stress research if the gap between such agronomic and ecophysiological experiments is to be bridged.

Further research is required to verify how multiple traits are involved in drought response in order to more efficiently develop improved crops for drought-prone environments. This study shows that the response of ecophysiological traits could not always be interpreted independently from the level of plant organization, which emphasizes the importance of the evaluation of drought response at the whole-plant level as a concerted response in different traits. High leaf-level responsiveness of wild *C. arabica* populations to soil moisture availability found in this study was not always paralleled by similar whole-plant responsiveness. Hence, phenotypic integration of ecophysiological traits deserves additional investigation and the incorporation of other taxa in the analysis is needed in order to improve the understanding of how complex phenotypes evolve.

In addition, the relationship between phenotype and fitness is mitigated by the environment in which organisms occur; thus, it is critical to relate phenotypic variation and variation in fitness to the environmental conditions experienced in nature. As this study shows, a high interaction with the environment and a response in one plant trait, leading into a fitness advantage under specific experimental conditions, could not replicated over contrasting environments, suggesting that the reliance on specific ecophysiological traits is only possible as long as the environment is appropriately simulated. In this study, the responses to varying levels of water and light availability can, therefore, not simply be extended to the conditions experienced by mature plants under natural environmental conditions. In the common-garden study, plants were exposed to progressive drought conditions that are not experienced *in-situ*. In the drought stress experiment, drought conditions developed rapidly, and the plants could not adjust through morphological changes by the time the soil dried. However, the results confirm the expected pattern under environmentally unpredictable conditions and suggest trends that deserve further exploration. Further studies, replicated across contrasting environments, are necessary to see if these results can be generalized.

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