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# Does geographic origin dictate ecological strategies in *Acacia senegal* (L.) Willd.? Evidence from carbon and nitrogen stable isotopes

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**Abstract**

*Background and Aims: Acacia senegal,* a leguminous dryland tree, is economically and ecologically important to sub-Saharan Africa. Water-use efficiency (WUE) and biological nitrogen fixation (BNF) are fundamental to plant productivity and survival. We quantify provenance differences in WUE, BNF, photosynthesis, biomass and gum arabic production from *A. senegal* assessing genetic improvement potential.

*Methods*: Using stable isotope ratios, we determined WUE ($\delta^{13}C$) and BNF ($\delta^{15}N$) from provenances of mature *A. senegal* in field-trials (Senegal), sampling leaves at the beginning (wet) and end (dry) of the rainy season. Seedling provenance trials (UK) determined photosynthesis, and biomass and $\delta^{13}C$ in relation to water table. Environmental data were characterised for all provenances at their sites of origin.

*Results*: Provenances differed in both $\delta^{13}C$ and $\delta^{15}N$. Gum yield declined with increasing WUE. Virtually no BNF was detected during the dry season and seedlings and mature trees may have different WUE strategies. Wind speed and soil characteristics at provenance origin were correlated with isotope composition and gum production.

*Conclusion*: Provenance differences suggest that selection for desirable traits, e.g. increased gum production, may be possible. As ecological strategies relate to native locality, the environmental conditions at plantation site and provenance origin are important in assessing selection criteria.

**Key words**

Water use efficiency; biological nitrogen fixation; sub-Saharan Africa; stable isotopes photosynthesis; light response curves; provenance

## Introduction

Water and nitrogen availability are major determinants of plant production in natural, semi-natural and agricultural ecosystems (Bacon 2004; Sprent 1987; Unkovich et al. 2008; Werner and Newton 2005). To a large extent, the structure of plant populations, communities, and phenology is determined by the spatial and temporal distributions and availability of water and nitrogen (N) in the soil (Bacon 2004; Do et al. 2005; Werner and Newton 2005). Both biological nitrogen fixation (BNF) and water-use efficiency (WUE; the ratio of photosynthetic production to the rate of transpiration) can be intrinsic physiological characteristics for species and there is also evidence for intra-specific variation in both parameters (Bacon 2004; Werner and Newton 2005). It is likely that trade-offs exist between the benefits and costs of, for example, BNF and WUE;) in relation to plant productivity and survival. This may be particularly evident in dryland systems where the temporal and spatial variability of N and water vary greatly. N availability can enhance biomass production, and selection for WUE may have practical implications in dryland regions where there are constraints on productivity (Ripullone et al. 2004). Hence, the detection of genotypes that maintain plant productivity and optimise WUE and BNF in different environmental conditions is an important (though particularly challenging) goal especially in developing countries (Bacon 2004; Werner and Newton 2005) where livelihood is closely coupled to ecological benefit.

Soil water availability is a pre-requisite for BNF to occur, and is particularly crucial in the drylands where soil type and climate largely determine the temporal and spatial variability of soil water, and thus the occurrence of BNF. As such, the temporal and spatial variability of BNF is not always clear and it also may not always be the dominant process by which plants obtain N; plants are equally likely to source N from different N cycling process, e.g.

recycling of nitrogen from deeper layers in interstitial waters or from decomposing tissues (Dupuy and Dreyfus 1992; Sprent 1987).

In dryland ecosystems, increased WUE is a potentially valuable trait which might be expected to be under strong selection pressure and hence highly heritable. Though more than one strategy may be evident, plants might be perpetually conservative in water use, or have a high water use and/or productivity in the wet periods before water stress conditions return. These strategies will depend on environmental factors and their temporal and spatial variability. Nevertheless, the potential for tree improvement would also be correspondingly high if improved genotypic/phenotypic WUE traits could be identified in species and associated with BNF. This would represent an ideal case where there is the potential not only to increase productivity but also to improve sustainability of land-use systems. Quantitative trait loci (QTL) analysis and genetic engineering are beginning to disentangle the genetic complexity surrounding adaptation to environmental conditions in crops (e.g. wheats), where it has been possible to enhance biomass production although this is mainly through improved soil water extraction efficiency than WUE *per se* (Tuberosa 2004). In *Quercus* spp. differences of $\Delta^{13}C$, (in bulk leaf matter, wood and cellulose) and intrinsic water use relate more readily to differences in stomatal conductance and stomatal density than photosynthetic efficiency (Roussel et al. 2009). Different WUE strategies exist between plants with different photosynthetic pathways (C3; C4; CAM) but also there is increasing evidence that they exist between and within species (Deans and Munro 2004; Gebrekirstos et al. 2011; Hausmann et al. 2005). These differences can also be broadly maintained irrespective of season (Deans and Munro 2004). Thus, the identification of optimal genotypes particularly when they are native legumes is a priority for dryland areas in Africa. Sprent et al. (2010) suggest that with modern techniques for improving both plant and rhizobial germplasms, better exploitation of native legumes is an achievable goal in Africa.

*Acacia senegal* (L.) Willd., is arguably one of the most important sub-Saharan African trees, inhabiting savannah systems that are under threat of anthropogenic and climate-mediated degradation and that have seen substantial losses of habitat (Hejcmanova et al. 2010; Obeid and Seifeldi 1970). The resolution of the taxonomic debate surrounding the delimitation of a type for *Acacia* has seen the re-naming of the African Acacia's and *A. senegal* to *Senegalia senegal* (L.) Britton. However, we have retained the use of *A. senegal* here for continuity with previous literature but more importantly because of the widespread familiarity of the African people with the name *Acacia* rather than *Senegalia*. *A. senegal* is a multi-purpose tree, producing 'gum arabic' (an economic staple in sub-Saharan regions) and used for animal fodder, multiple timber products, intercropping, firewood, food and medicines (see Fadl and El Sheikh 2010; Fagg and Allison 2004; Sprent et al. 2010). The collection and sale of gum arabic can be especially important as it is produced in the dry season when other income is limited (Fagg and Allison 2004) and it is possible for families to earn up to $150 per month during this season. Phylogeographic studies suggest that *A. senegal* var. *senegal* has undergone some past fragmentation in its West African range offering the potential for provenances to have built up some adaptive differentiation (Odee et al. 2012). This may have arisen during parallel cycles of expansion and contraction of the lowland rainforest in response to climate change during earlier epochs (Maley 2001; Plana 2004). As a legume, it is important for BNF and is associated with nodulating rhizobial bacteria from mostly fast-growing genera *Rhizobium* and *Sinorhizobium* (Fall et al. 2008; Nick 1998; Odee et al. 2002). The improved nutrient status in the topsoil beneath trees that associate with nitrogen fixing bacteria is well documented (Näsholm et al. 2009; Sprent et al. 2010; Werner and Newton 2005). Although some research is beginning to point to the existence of variation between provenance genotypes within *A. senegal* (see e.g. Raddad and Luukkanen 2006), the goal of identifying optimal ecotypes or genotypes for any given environment requires much further research.

Differences in the ratios of stable isotopes in plant foliage are often used as surrogates for the quantification of BNF [$^{15}N$/ $^{14}N$, abbreviated as $\delta^{15}N$] and to reflect overall plant water use [$^{13}C$/$^{12}C$, abbreviated as $\delta^{13}C$] (Farquhar et al. 1989). Here we present data on N and C isotope ratios for *A. senegal* to build on the current evidence base (Fagg and Allison 2004; Raddad et al. 2005; Raddad and Luukkanen 2006) and provide insights into existence of ecotypes (a genetically distinct geographic variety) within *A. senegal* at the provenance level. We examine WUE ($\delta^{13}C$), BNF ($\delta^{15}N$) in provenance trials in Senegal and glasshouse trials to quantify:

1. between provenance variation in :
    a. WUE using leaf level $\delta^{13}C$ as a surrogate for WUE;
    b. Estimates of BNF using leaf levels of $\delta^{15}N$;
    c. biomass allocation and photosynthetic characteristics.
2. within provenance variation in:
    a. WUE using leaf level $\delta^{13}C$;

and

3. relate variation in WUE and BNF to the environmental variables of the 'native' range of each provenance.

## Materials and Methods

### *Senegal Trials*

We used two provenance trials of *A. senegal* var. *senegal* (containing the same provenances from across much of the natural range of the species, but grown under different environmental conditions) that were set up in 1994 by Institut Sénégalais de Recherches Agricoles (ISRA) and Centre National de Recherche Forestières (CNRF) at Dahra and Bambey, Senegal (Figure 1 and Table 1). At both sites, a randomized block design of four blocks was used, within which 17 randomly assigned provenance plots were established, each containing twenty five randomly chosen plants from that provenance, planted at 5 m spacing. Note that a subset of 10 these provenance was used for sampling (see below) Bambey has soils typically composed of leached tropical ferruginous sandy clays, with a mean rainfall of around 400 mm $yr^{-1}$ and a rainy season extending from June to September. Dahra's dry *Acacia* woodlands are typically open to grazing, and on leached, tropical ferruginous soils (soil chemistry is detailed in Ndoye et al. 2012) ; it has a mean rainfall of around 250 mm $yr^{-1}$ and a slightly shorter rainy season from July to September. We concentrated most of our sampling on Dahra which is within the natural range for gum production, whereas wetter Bambey is not. Seeds for these trials had been collected using standard protocols (see e.g. FAO 1993) and trees were not inoculated prior to planting out at the sites. Tree height data is given in Table S1 in the supplementary material.

### *Selection of Provenances*

Provenance trials have been used in forestry since the early 19th century for detecting populations with desirable characteristics for tree-breeding programmes (Guries 1990). The benefit of these 'common-garden' type studies, is that environmental conditions are controlled, and quantitative traits easily scored (Johnson et al. 2004). Despite the fact that most trees are long lived, even seedling studies are justifiable since selection in trees is thought to operate most efficiently at very early developmental stages (Persson and Ståhl 1990; Petit and Hampe 2006). Thus such trials are the ideal experimental design for the detection of differences across provenances and are extremely important for studies of adaptation to climate change (Aitken et al. 2008; Kawecki and Ebert 2004; Mátyás 1996). For species such as *Acacia senegal* that is highly likely to show a wide plastic response, provenance trials allow the partitioning of variation from different sources. Here, we take plasticity to be defined as the capacity of a single genotype (provenance) to exhibit variable phenotypes in different environments (*sensu* Bradshaw 1965) and, so variation in the phenotype may be expressed as:

$$VP = VG + VE + VG*E + V\mathrm{error}$$

Where: $VP$ = Total phenotypic variance for a trait;

$VG$ = Genetic variance (proportion of phenotypic variation attributable to genes);

$VE$ = Environmental variance (proportion of variation caused by the environment);

$VG*E$ = Genotype x Environment interaction (Genetic variation for phenotypic plasticity);

*Verror* = Unexplained variance, including developmental noise, measurement error, etc.

When the plants are grown in common environment trials $VE$ and $VG*E$ are minimised thus the remaining phenotypic variation is largely due to $VG$. Whilst there is undoubtedly a degree of plasticity, provenance level differences were assessed on the residual variation after the site, season and block were removed. Thus we can assume any provenance level detected is genetically controlled (see analysis below).

Selection of provenances for this study was based on gum yield data from both sites (for 2001 and 2007-9). From all surviving trees at each time period we calculated a mean for each provenance and an abundance weighted mean. These were weighted according to the proportion of trees in each provenance contributing to the gum production rather than all surviving trees, i.e. where there are few trees contributing to the production values this down-weights the resulting mean. However, both of these mean values were highly correlated (Pearson's r = 0.94) and gave the same qualitative results. On the basis of this data, we selected ten provenances, ranked from low to high yielding (Table 1 and Figure 1).

*Sampling for Stable Isotope Studies*

We targeted our sampling towards the beginning (wet) and end (dry) of the rainy season in August and October 2009. Note that although we use the terminology 'dry season' here October is not really the dry season *per se* but the beginning of the dry season. This was for two reasons: firstly, we could not sample in the dry season since *Acacia senegal* sheds most of its leaves during this period: secondly, gum tapping is traditionally timed to occur when around half the leaves have fallen from the canopy towards the end of the rainy season/beginning of the dry and hence represents a crucial time period in the interpretation of our results. Site level differences in the August data can  be examined in terms of differences in soil water since Bambey is a much wetter site and is  located outside the zone of gum production and therefore allows a similar dry/wet comparison to the seasonal change.

At each site, leaf material was collected from the ten *A. senegal* provenances in each of four experimental blocks, in addition, reference plant material was also collected (see below); in total 200 samples were collected. For the August sampling campaign (wet season) all chosen provenances at both sites were included. In October 2009 (dry season) only the drier Dahra trial was sampled using a subset of the provenances analysed in the August campaign, namely Kordofan, Kankoussa, Kirane, Diaménar, Karofane and Ngane. At each harvest, five trees from each of the selected provenances in each block were randomly chosen from which mature fully expanded sun leaves were collected; the same trees were harvested during both wet and dry sampling periods. Field samples were collected into porous paper bags to allow drying while preventing loss of pinnules. Thirty leaves for $\delta^{13}C$ and 20 leaves for $\delta^{15}N$ were bulked for isotope analysis for each tree. The dry season sample for one Kankoussa tree was lost and so this tree was eliminated from the analysis making 19 trees available for analysis. Additionally, from the Kankoussa provenance ($\delta^{13}C$ only), again 5 trees were selected per block but 5 (wet season) and 10 (dry season) individual leaves were harvested from each tree and analysed separately to give some measure of within tree $\delta^{13}C$ variability. The height of all the sampled trees was estimated using a Haglöf (Långsele, Sweden) HEC electronic clinometer.

$\delta^{13}$ C is negatively associated with water use efficiency over the period of dry mass production and under drought stress it is a good predictor of stomatal conductance at least for crop species (Condon et al. 2004). Thus, we interpret low $\delta^{13}C$ values as indicative of high WUE and high $\delta^{13}C$ values as indicative of low WUE.

In order to estimate BNF, $\delta^{15}N$ was also analysed from leaves of non-$N_2$-fixing reference species (i.e. from species that have no known BNF capability) occurring within the vicinity of the trial sites. As these species were not explicitly part of the experimental trials, sampling was on the edges of blocks and as would be expected, herbaceous plants were only available in the early wet season. Consequently, the non-$N_2$-fixing reference plants were variably sampled depending on availability. They were identified and grouped as follows: trees, shrubs, herbs and grasses.

The $^{15}N$ natural abundance method estimates nitrogen derived from the atmosphere (Ndfa) as the contribution of nitrogen in putative $N_2$-fixing legumes which originates from BNF, calculated according to Shearer & Kohl (1986) using the following equation:

$$\%Ndfa = (\delta^{15}N_{ref} - \delta^{15}N_{leg})/(\delta^{15}N_{ref} - \delta^{15}N_{fix})100;$$

(where $\delta^{15}N_{ref}$ is $\delta^{15}N$ of non-fixing reference plants; $\delta^{15}N_{leg}$ is $\delta^{15}N$ of the putative $N_2$-fixing plant; $\delta^{15}N_{fix}$ is $\delta^{15}N$ of fixed $N_2$). For $\delta^{15}N_{fix}$, a value of -2.52 ‰ of *A. senegal* seedlings grown in N-free environment and solely dependent on BNF was used as reported by Isaac et al. (2011). Of all the non-$N_2$-fixing reference plant species, only *Balanites aegyptiaca* (L.) Del. had higher mean $\delta^{15}N$ than *A. senegal* for samples collected during the wet season. Thus, BNF was estimated during the wet season using *B. aegyptiaca* as non-$N_2$-fixing reference ($\delta^{15}N_{ref}$) plant. The species has previously been used as a reference plant in estimating BNF in natural populations of *A. senegal* (Isaac et al. 2011). *B. aegyptiaca* is also a tree, which remains green all year and will have a more similar root architecture to *A. senegal* than herbaceous species.

For stable isotope analysis, a portable microwave oven (c.f. Arndt and Wanek 2002; Popp et al. 1996) was used to rapidly dry the leaves (Wavebox portable microwave, Model No. WBP-TP-660, output 660 w). We conducted a pilot test comparing conventional oven drying and the microwave technique using *Rumex acetosa* L. leaves. These leaves were split along the mid-rib, with one half oven dried and the other microwave dried. There was no difference in $\delta^{13}C$ values between oven and microwave dried leaves when instrumental error was taken into account (supplementary material, Figure S2). After drying, leaves were stored in paper bags inside air-tight plastic bags or boxes with self-indicating silica gel for transport.

*Glasshouse Trials*

Two common environment trials were established in a semi-controlled glasshouse environment in the UK, (conducted over the UK summer period June –August) set up to mimic tropical conditions (mean temperature 25 °C, maximum 35 °C, minimum 18 °C; mean relative humidity 65% maximum 95% and minimum 30%). We used seeds of several provenances sourced from the World Agroforestry Centre (ICRAF) and wild collections (Fig

1a and Table 1). These covered the entire ecological range represented by the provenances used in the field trials in Senegal, but were not identical as the original seedlots were no longer available. The glasshouse trials enabled photosynthetic light curves to be determined under controlled conditions and water table effects on seedling development and biomass allocation to be evaluated. Seed was pre-screened for any surface deformity before being randomly allocated to treatments. To enhance germination, seeds were pre-treated by rubbing a small area of seed coat opposite the micropyle with fine sand paper and soaked in cold water until the seed had swelled.

*Light Curve Determination*

A randomized block design trial was set up in 2009 to estimate photosynthetic light curves for four month old saplings from 12 provenances. Eight blocks were set up with one tree per provenance in each block and light curves were estimated from one mature leaf per tree giving a total of ninety six light curves i.e. eight curves per provenance. However, data for three light curves were rejected- Bissiga (2) and Kigwe (1) due to a leaky chamber, which led to an unbalanced design for analysis (see below). Instantaneous WUE was also calculated as the ratio of evapotranspiration to carbon fixation. Light curves were determined using an LI-6400XP portable photosynthetic system (LICOR Environmental - UK Ltd, Cambridge, UK) and light was supplied by an internal red/blue LED light source (LI6400-02B). The leaf temperature was controlled at 28 ºC; the maximum deviation was near 2 ºC but was usually less than 1 ºC during the course of curve determination. Ambient air was scrubbed of $CO_2$, and $CO_2$ was controlled by addition from pressurized bottles at around 400 ppm; vapour pressure deficit (VPD) was kept constant at 1.8 kPa. Light response curves were generated using the LICOR photosynthesis software equations in LICOR application note 105 (Norman et al. 1992). Leaf area was determined from photographs of the leaves in the chamber, analysed in Image J software (Rasband 1997-2011). Instantaneous WUE was calculated from the light curve data generated from PAR values ranging from 2000 µmol $m^{-2}$ $s^{-1}$to 0 µmol $m^{-2}$ $s^{-1}$.

*Water Table Effects*

A second randomized block design trial was set up in 2010 using the same 12 provenances planted in 10 cm diameter pots of 30 cm and 50 cm depths, representing two different depths to water source. There was one replicate of each provenance × depth combination in each of eight blocks. All pots were located on a bench with watering system set up to soak capillary matting for 2 minutes 30 seconds every 2 hours from 07.00 - 18.00 h. Pots were filled with John Innes number 3 compost, with 3 cm of seed compost on top. Seeds were sown (on 1st June 2010) on the surface (one per pot) and covered lightly with horticultural sand. After seven weeks plants were destructively harvested and scored for survival, and measurements made of root and shoot biomass; this material was dried in a conventional oven at 60 ºC for 24 hrs. Leaf area and specific leaf area were estimated from a random sample of 3 mature leaves per tree. These leaves were then bulked for each provenance to determine $\delta^{13}C$ value. Plants that failed or struggled to reach the water source (as indicated by either low root biomass or by dying before harvest) typically showed symptoms of water stress e.g. leaf loss.

*Laboratory Analyses*

Dried leaf samples were ground by a ball mill to a fine powder and analysed at two isotope laboratories, the Life Sciences Mass Spectrometry Facility (LSMSF) at Lancaster, UK (for $^{13}C$ analysis) and the Stable Isotope Facility (SIF) at UC Davis, California, USA mainly for $^{15}N$ but additional $^{13}C$ data were also provided by this laboratory and used in the analyses. At the LSMSF laboratory, $^{13}C$ analysis samples were weighed into tin capsules and combusted using a CarloErba elemental analyser. Resultant $CO_2$ from combustion was analysed for $\delta^{13}C$

using a Dennis Leigh technology isotope ratio mass spectrometer (IRMS). At the SIF, weighed samples in tin capsules were analysed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were combined with several replicates of at least two different laboratory standards previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). The long term standard deviation is 0.2 ‰ for $^{13}C$ and 0.3 ‰ for $^{15}N$. All values were expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and air for carbon and nitrogen, respectively.

*Statistical Analysis and Environmental Characterisation of Provenances*

As our sampling was unbalanced (i.e. 5 leaves in the wet and 10 in the dry), for the Senegal trials, we applied an unbalanced analysis of variance to the data using GenStat (version 13, VSN International Ltd., Hemel Hempstead, United Kingdom).

We also included in our analysis an assessment of life-form mainly to interpret BNF difference between fixing (Acacia) and non-fixing (all the other species) but also in WUE since there are likely to be differences in life form water use strategies. The life-forms used here are as follows: leguminous trees (*Acacia*) non-leguminous trees (trees), shrubs and herbaceous plants (herbs).

To account for any block effects tests for site, season, life-form and provenance effects were done once block effects had been accounted for i.e. on residual variance. In addition, provenance effects were only tested once variation that could be attributed to plasticity was removed (site, block and season). As reference material was collected outside the trial and to remove variation due to this these were treated as an additional block in the analysis (block 5). The growth form factor used the classification *Acacia*, (i.e. *Acacia senegal* only), tree (all other tree species), shrub (woody species < 5m), herb and grass. For the glasshouse studies a balanced analysis of variance was applied to the data again using GenStat and testing for any effects once the block effects were accounted for. For the Senegal data both tree height and laboratory ($\delta^{13}C$ only) were initially included as covariates in the full model but were subsequently dropped from the analysis as they were found not to be significant.

To examine the links between the $\delta^{13}C$ and $\delta^{15}N$ provenance results and 'native' environmental conditions i.e. those at the place of origin of the different provenances, not those at the trial sites, we used summary climate data from LocClim (see Gommes et al. 2004) and soil data from The Harmonized World Soil Database (HWSD) (FAO/IIASA/ISRIC/ISSCAS/JRC 2009) (see supplementary material, Table S2, for the variables included). These data were subjected to Principal Component Analysis (PCA) in Canoco 4.5 (ter Braak and Smilauer 2002). The isotope data were entered into the PCA model as supplementary variables. These are passively entered into the ordination but do not influence the ordination axes i.e. they are added post-analysis so that their relation to the other variables can be judged from the ordination diagram (see ter Braak and Smilauer 2002). Correlation analysis was also performed to determine the relationship between the traits.

## Results

### *Senegal Trials*

### *Provenance $\delta^{13}C$*

Block was a significant term in the model, but analysis once this was accounted for, revealed clear effects of season, site, life-form and provenance in terms of $\delta^{13}$C (Table 2). $\delta^{13}$C values were lower in the dry season compared to the wet and values from the trial at Dahra tended to be lower than those from Bambey (Figure 2). There were also differences in life-form with *Acacia* tending to show lower values than herbs, shrubs or other tree species, particularly in the dry season. Provenance level differences were more evident in the dry season and $\delta^{13}$C values correlated with gum production: lower values were correlated with higher gum production (Figure 2). This relationship appeared to be stronger in the dry season although sample size was lower in the dry season data.

### *Provenance $\delta^{15}N$ and %N*

Again, although block was a significant term in the model, there were similar seasonal, site, life-form and provenance effects for $\delta^{15}$N values (Table 2). $\delta^{15}$N values were lower in the wet season compared to the dry and values from the trial at Dahra tended to be slightly higher than those from Bambey (Figure 3). Life-form also shows differences with *Acacia* tending to have higher values in the dry season but similar values to herbs and other trees in the wet season. Provenance level differences were more evident in dry season. In contrast to the $\delta^{13}$C data there was no correlation between gum production and $\delta^{15}$N (Figure 3). Results showed high variability of $^{15}$N natural abundance values within and among provenances with a significant effect ($P < 0.05$) of season (dry & wet) and location of the experiment (Dahra & Bambey). At Dahra, the $\delta^{15}$N values among all provenances (pooled) ranged from 2.33 ‰ to

11.22 ‰ during the dry season and from 4.45 ‰ to 8.23 ‰ during the wet season. The $\delta^{15}N$ values for reference plants ranged from 3.86 ‰ (*Combretum glutinosum* Perr., tree) to 6.62 ‰ (*B. aegytiaca,* tree) for dry season and from 3.16 ‰ (*Bauhinia rufescens,* Lam., shrub) to 8.47 ‰ (*Achyranthes argentea*, herb) for wet season. At Bambey, where only wet season samples were analysed; the $\delta^{15}N$ values ranged from 1.33 ‰ (*A. senegal*, Kirane provenance) to 8.47 ‰ (*A. senegal*, Sudan provenance [=Kordofan]) . All provenances showed lower $\delta^{15}N$ values at Bambey (3.81 ‰ to 5.05 ‰) than Dahra (6.11 ‰ to 6.41 ‰). The $\delta^{15}N$ values for reference plants ranged from 2.13 ‰ (*Zizyphus mauritiana* Lam., shrub) to 7.32 ‰ (*B. aegyptiaca,* tree) . $\delta^{15}N$ values for the grasses, *Brachiara disticophylla* Stapf. and *Andropogon gayanus* Kunth., sampled during the wet season in Bambey were 4.29 ‰ and 4.99 ‰, respectively. In Dahra, the mean % N values for *A. senegal* provenances were generally similar for both wet (4.2 % -4.6 %) and dry (4.1 % - 4.4 %) season. Reference plants showed slightly higher mean % N values in wet than dry season (4.0 % vs. 3.3 %). In Bambey, mean % N values ranged from 3.4 % to 4.3 % for *A. senegal* provenances, while that of reference plants was significantly lower (2.4 %).

In order to be able to estimate BNF by the $^{15}N$ natural abundance method, one of the most important criteria is that $\delta^{15}N$ values of the putative $N_2$-fixing plant ($\delta^{15}N_{leg}$) are between $\delta^{15}N_{fix}$ and $\delta^{15}N_{ref}$ (Shearer & Kohl, 1986). Among all the analysed reference plants, only *B. aegyptiaca* could be reliably used as a suitable non-$N_2$-fixing reference plant ($\delta^{15}N_{ref}$) during the wet season. In Bambey, the only sampled *Balanites aegyptiaca* tree had $\delta^{15}N$ value of 7.32 ‰, while the nearest *A. senegal* provenance (Kordofan) had a mean $\delta^{15}N$ value of 5.07 ‰, indicating a difference of > 2 ‰ between the putative $N_2$-fixing and non-$N_2$-fixing reference plants (Table S3). In Dahra the difference in $\delta^{15}N$ values between *B. aegyptiaca* and *A. senegal* was < 1 ‰ during the wet season, while *A. senegal* provenances were more enriched than any of the reference plants in the dry season. Estimates of BNF based on the wet season data with *B. aegyptiaca* were either low or negligible in Dahra (< 15 %), with Sodera (Ethiopia) provenance showing virtually no fixation. On the other hand, BNF estimates in Bambey ranged from 28 % for Ngane provenance to 38 % for Kankoussa provenance.

*Variation of $\delta^{13}C$ Abundance Within and Among Kankoussa Provenance Trees*

As predicted, the block effect was also present within the Kankoussa $\delta^{13}C$ data (Table 2). However, once block effect was accounted for there were similar seasonal effects as in the whole provenance analysis as well as a significant effect of tree. Seasonal effects followed a similar pattern as above with wet season values tending to be greater than dry season (Figure 4). Among tree variability was as great as between provenances indicating that individual strategies also vary; one tree reversed the predominant trend having a higher mean $\delta^{13}C$ value in the dry compared to the wet season (Figure 4).

*Glasshouse Trials*

*Light Curves*

A range of mean light curve parameter values was evident (Table 3). However, only the response to light saturation showed significant variation among provenances (see supplementary material Table S4). The variation in maximum assimilation rate (Amax) was not significant (p=0.09) but is mentioned here as a significant correlation was detected (see below). Mean light saturation ranged from approximately 1100 μmol $m^{-2}$ (Fallatu Forest) to 2500 μmol $m^{-2}$ (Kibwezi and Ouagadougou). Mean maximum assimilation rates ranged from approximately 30 μmol $s^{-1}$ $m^{-2}$ (Mali) to 60 μmol $s^{-1}$ $m^{-2}$ (Ouagadougou) (see Table 3 and correlations with biomass below).

*Water Table*

In the 30 cm pots Kibwezi had the largest mean leaf area (and standard error) while Bissiga had the largest specific leaf area (Table 4). Ouagadougou had the smallest leaf area although this was from one tree (the other surviving tree had no leaves). Dara had the largest mean root biomass but both India ICRAF and Kigwe had more than double the shoot biomass of the other provenances showing distinct provenance differences. A negative correlation was noted between Amax and survival in 30 cm pots (R= - 0.63; p = 0.027). Cameroon had the highest level for instantaneous WUE and Kiembara the lowest but this measure did not correlate to any of the other estimated values including $\delta^{13}C$.

In the 50 cm pots India ICRAF had the largest leaf area and again Ouagadougou had the lowest but again this was based on only one tree. Again, Bissiga had the largest specific leaf area but in contrast to the 30 cm pots India ICRAF had the largest root and shoot biomass. Also in common with above both India and Kigwe had shoot biomass more than double that of the other provenances. A negative correlation with light saturation and survival in 50 cm pots (R= -0.63; p= 0.028) was also detected.

There was no detectable difference between pot treatments in bulked provenance $\delta^{13}C$. However, there was a significant correlation between the amount of biomass and the $\delta^{13}C$ in the 50 cm pot but no correlation in the 30 cm pots (Table 4). None of the estimated leaf variables measured correlated with $\delta^{13}C$ and there were no differences in leaf area or specific leaf area between pot treatments (data not shown). However, root biomass was positively correlated with $\delta^{13}C$ in 50 cm pots (R=0.84; p=0.005), and light saturation was positively correlated with $\delta^{13}C$ in 30 cm pots (R=0.84; p=0.002).

*Environmental Characterisation of Provenances*

The PCA ordination showed some potential links to environmental characteristics from provenance home locations (Figure 5). $\delta^{13}C$ value positively correlated with wind speed and some soil characters, particularly organic matter (Top OM, Figure 5) and water content (AWC, Figure 5). Variation in $\delta^{13}C$ values from the glasshouse experiment and field trial in Senegal were in opposing directions. Light saturation was negatively correlated with irradiance (R= -0.63; p= 0.027) and potential evapotranspiration (R= -0.63; p= 0.028) and positively correlated to rainfall (R=0.67; p=0.017).Variation in wind was also negatively correlated with weighted mean gum production (R=-0.85; p=0.033). $\delta^{15}N$ was negatively correlated to some soil characteristics particularly bulk density and climate in terms of air temperature. Figure 5 would also suggest that water content and potential evapotranspiration may also be important factors for $\delta^{15}N$.

## Discussion

### *Water Use Efficiency*

We used $\delta^{13}C$ as a surrogate for WUE and found clear provenance level differences and significant within provenance variation. In combination with the results of PCA, this demonstrates a genetic basis for eco-physiological differences among and within provenances, probably as a result of adaptation to local environments at their geographic sites of origin. While, Raddad and Luukkanen (2006) found that values of $\delta^{13}C$ were less negative in the *A. senegal* provenances from sandy soils compared to those from clay soils. This suggests that provenances from sandy soils displayed conservative water use. Similarly, Newton et al. (1996) found that *Acacia tortilis* (Forssk.) Hayne trees growing on drier sites displayed relatively higher WUE than those on a wetter site. Likewise, Midgley et al. (2004) found evidence to suggest higher leaf-level WUE in dominant woody species (including *Acacia* spp.) from drier savannah sites. As such, our results add to the growing evidence that variation in WUE occurs within species as well as between species.

In addition, we show that *A. senegal*, may exhibit different WUE strategies at different life stages. Results from our glasshouse trial on young plants concur with Raddad & Luukkanen (2006), in that provenances from sites with low water availability showed conservative water use. Whilst our results from the mature trees in the Senegal trials suggest trees from dry sites were less water use efficient, they are in contrast to reports of other studies with *Acacia* and woody species in dryland savannas (e.g. Newton et al. 1996; Midgley et al. (2004). Although experimental artefacts cannot be ruled out, it is possible that the *A. senegal* has a plasticity in WUE that maximises water use efficiency whilst young and shallow-rooted, but relaxes water control once roots are deeper and able to access groundwater (Ong et al. 2002; Otieno et al. 2005a; Otieno et al. 2005b). Nonetheless, field comparisons will be required to compare mature and young trees in common environments to establish if this observation is robust (c.f. Gebrekirstos et al. 2006).

Interpreting foliar $\delta^{13}$ C in terms of plant water use efficiency (WUE) relies on the assumption that it is stomatal conductance and not carbon assimilation that varies. We have no direct evidence of whether the provenance differences in WUE are a result of stomatal conductance or carbon assimilation, as our $\delta^{13}$C values did not correlate with instantaneous WUE. Departures from the relationship have been reported (Marron et al. 2005; Monclus et al. 2006; Monneveux et al. 2006), however, our photosynthesis measurements were made on different plants and at a different time period to those for $\delta^{13}$C. Ultimately WUE at the leaf level depends on stomatal regulation, evaporative demand and carbon fixation. WUE and $\delta^{13}$C can vary independently (Seibt et al. 2008) because as stomata respond, depending on whether there is isohydric (tight stomatal control, resulting in a minimum, threshold leaf water potential for stomatal closure) or anisohydric (less strict control, with no discernible threshold) responses, the influence of vapour pressure deficit is modified such that further increases in vapour pressure deficit after a certain value do not influence WUE (Maseda and Fernández 2006).

We also found differences in $\delta^{13}$C values that correlate with gum production. The Sudanese provenance produced the most consistent gum supply and was characterised by a lower $\delta^{13}$C signal. This largely agrees with the findings of Raddad & Luukkanen (2006) who found a similar relationship with gum yield. However, Gum yield is known to vary according to a number of factors such as climate and tapping date and method (Ballal et al. 2005a; Ballal et al. 2005b; Raddad et al. 2006). Reliable data on gum arabic yield and gum yield trends are generally lacking (Ballal et al. 2005a and references therein), therefore, more comprehensive datasets and detailed examination are required before firm conclusions can be drawn.

*Biological Nitrogen Fixation*

Estimates of BNF by *A. senegal* during the wet season were within the range reported for the species in a four year-old provenance trial in the Blue Nile region, Sudan (cf. 24-61%, Raddad et al. 2005), and comparable to those from natural populations of *A. senegal* at Baringo, Kenya, in the Rift Valley (cf. 33 -39%, Isaac et al. 2011). However, our estimates, particularly in Bambey, should be treated with caution because they were based on a single non-$N_2$-fixing reference tree, *B. aegyptiaca*, which had a $\delta^{15}N$ value higher than the mean values of the *A. senegal* provenances (Table S3). The $\delta^{15}N$ values of the rest of the non-$N_2$-fixing reference plants (other trees, shrubs and herbs), including their means, were indistinguishable from $\delta^{15}N$ values for *A. senegal* (Figure 3). The $\delta^{15}N$ values of *A. senegal* trees at Dahra during the dry season were highly enriched, and mostly surpassed those of the reference plants, resulting in negative or non-significant BNF estimates (Table S3). Similar null estimates have been observed in natural *A. senegal* trees, which were attributed to low P soils (Isaac et al. 2011), and, in *Acacia* species in dry woodland savannas, constraints on water availability (Handley et al. 1994). In our study, high temperatures and moisture stress may be responsible for the null estimates at Dahra during the dry season. Such conditions are common during dry seasons in the Sahelian region and may constrain legume root-nodule formation and function (Hungria and Vargas 2000; Rasanen and Lindstrom 1999).

However, it is also possible that, particularly in the dry season, there is an alternative N source available. Although soil was not analysed in this study, the high and variable leaf N concentrations in both putative $N_2$-fixing *A. senegal* provenances and non-$N_2$-fixing reference plants suggest different sources of plant available soil N. As suggested for the water relations above, the mature trees may tap into the widespread N source below the water table found in the interstitial waters in this region (Deans et al. 2005; Edmunds and Gaye 1997). *Acacia* species are certainly known to access water resources from this depth (Otieno et al. 2005b), and may well make use of available N at the same time. Alternatively, organic matter may be a possible source (see Näsholm et al. 2009). For example, Bernhard-Reversat & Poupon (1980) found mineralization of organic N in the topsoil to be relatively important directly under trees suggesting N cycling to be an important source of plant available N, with the process occurring at the beginning of the rainy season.

*Eco-physiological Strategies in* Acacia senegal

Our results indicate that variation in $\delta^{13}C$ and $\delta^{15}N$ in *A. senegal* has both genetic and age-related components. Among and within provenance differences, probably arise as a result of adaptation to local environments in the region of origin. This suggests that selection for gum production in *A. senegal* might also be successful; it is certainly likely that some passive selection has already occurred particularly in Sudan populations. However, both plantation and provenance location environmental conditions are likely to be important in assessing selection criteria. In addition, our results suggest that high gum yield is associated with plants that have lower WUE and so there is potential for conflict in selection criteria. Experience has shown that improvements in leaf-level water use efficiency may not always translate into higher crop water-use efficiency or yield (Condon et al. 2004). Nevertheless, the level of environmental variability, the between and within population variation reported here and the differences in age class, suggest that there are prospects for genetic improvement. Given *A. senegal's* potential poverty alleviation importance, especially in the dry season, this should be a future research priority.

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**Tables**

Table 1: Origins and environmental characteristics of *Acacia senegal* provenances used in the field and glasshouse studies S = Senegal trial; G= Glasshouse Trial; * indicates wild collected seed. Gum yield data are shown for provenances for which they were available (based on data for 2001). PET = potential evapotranspiration.

| Country | Provenance | Longitude (decimal degrees) | Latitude (decimal degrees) | Alt (masl) | Mean monthly rain (mm) | Mean monthly PET (mm) | Mean Temp (°C) | Exp. Trial | Mean Provenance gum Prod. (g $yr^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Sudan | Kordofan | 29.58 | 12.73 | 620 | 32.0 | 205.0 | 26.0 | S | 244 |
| Mali | Aïte | -11.65 | 15.08 | 80 | 45.6 | 151.8 | 30.3 | S | 194 |
| Mauritania | Kankoussa | -11.45 | 15.93 | 80 | 27.2 | 179.2 | 29.5 | S | 190 |
| Chad | Tourba | 15.3 | 12.82 | 280 | 29.3 | 172.5 | 29.9 | S | 156 |
| Ethiopia | Sodera | 39.38 | 8.4 | 1500 | 64.8 | 114.5 | 20.7 | S | 136 |
| Mali | Kirane | -10.25 | 15.38 | 140 | 25.5 | 203.8 | 28.9 | S | 127 |
| Senegal | Diaménar | -15.9 | 16 | 20 | 25.2 | 197.2 | 25.4 | S | 105 |
| Niger | Karofane | -6.18 | 14.3 | 280 | 44.3 | 163.7 | 27.4 | S | 94 |
| India | Indie 50/60 | 79.52 | 26.32 | 120 | 87.1 | 146.4 | 25.8 | S | 91 |
| Senegal | Ngane | -16.2 | 14.13 | 0 | 49.3 | 170.2 | 28.0 | S | 79 |
| Burkina Faso | Bissiga | -0.53 | 12.43 | 280 | 62.3 | 181.9 | 28.5 | G* | |
| Cameroon | Cameroon | 14.23 | 10.25 | 440 | 65.8 | 190.8 | 27.9 | G* | |
| Burkina Faso | Dara | -0.18 | 14.33 | 260 | 32.4 | 157.9 | 28.9 | G* | |
| Sudan | Fallatu Forest | 30.14 | 13.1 | 600 | 26.5 | 205.0 | 27.3 | G | |
| India | India ICRAF | 73.14 | 26.72 | 260 | 33.5 | 159.3 | 26.5 | G | |
| Kenya | Kibwezi | 38.07 | -2.21 | 700 | 56.2 | 126.1 | 24.0 | G | |
| Burkina Faso | Kiembara | -2.72 | 13.25 | 300 | 49.0 | 160.4 | 28.3 | G* | |
| Tanzania | Kigwe | 35.48 | -6.1 | 940 | 44.7 | 154.3 | 23.6 | G | |
| Burkina Faso | Kirbou | -2.07 | 13.26 | 300 | 50.1 | 160.4 | 28.1 | G* | |
| Mali | Mali | -11.43 | 14.45 | 40 | 52.7 | 161.8 | 29.3 | G* | |
| Burkina Faso | Ouagadougou | 1.5 | 12.38 | 300 | 60.8 | 160.9 | 28.9 | G* | |
| Tanzania | Wangingombe | 34.63 | -8.85 | 1320 | 67.7 | 129.0 | 22.2 | G | |
| Senegal | Dahra Trial | -15.43 | 15.35 | 60 | 32.8 | 149.7 | 27.2 | | |
| Senegal | Bambey Trial | -16.47 | 14.71 | 20 | 42.8 | 176.0 | 26.5 | | |

Table 2: Results of $\delta^{13}C$ and $\delta^{15}N$ analyses of variation of water use efficiency and biological nitrogen fixation in *Acacia senegal*. The top two sections give results for $\delta^{13}C$ and $\delta^{15}N$ analysis of variance using an unbalanced design for the *A. senegal* leaf samples from the trials at Dahra and Bambey in Senegal. The bottom section is the results of a balanced analysis of variation for the $\delta^{13}C$ results for Kankoussa provenance at Dahra only.

| Variate | Factor | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|---|
| $\delta^{13}C$ | Block | 4 | 292.1 | 73.0 | 45.5 | <0.001 |
| | Season | 1 | 123.1 | 123.1 | 76.7 | <0.001 |
| | Site | 1 | 7.5 | 7.5 | 4.7 | 0.031 |
| | Life-Form | 2 | 75.6 | 37.8 | 23.5 | <0.001 |
| | Provenance | 10 | 34.0 | 3.4 | 2.1 | 0.021 |
| | Residual | 837 | 1343.8 | 1.6 | | |
| | Total | 855 | 1876.2 | 2.2 | | |
| $\delta^{15}N$ | Block | 4 | 83.6 | 20.9 | 11.6 | <0.001 |
| | Season | 1 | 351.5 | 351.5 | 194.7 | <0.001 |
| | Site | 1 | 163.8 | 163.8 | 90.8 | <0.001 |
| | Life-Form | 2 | 17.2 | 8.6 | 4.8 | 0.009 |
| | Provenance | 10 | 36.2 | 3.6 | 2.0 | 0.033 |
| | Residual | 304 | 548.7 | 1.8 | | |
| | Total | 322 | 1201.0 | 3.7 | | |
| $\delta^{13}C$ | Block | 3 | 6.9 | 2.3 | 7.9 | <0.001 |
| Dahra | Season | 1 | 36.6 | 36.6 | 125.5 | <0.001 |
| Kankoussa | Tree | 13 | 26.3 | 2.02 | 6.9 | <0.001 |
| | Residual | 267 | 77.9 | 0.29 | | |
| | Total | 284 | 147.8 | 0.52 | | |

Table 3: Mean photosynthesis parameters (SD) derived from light curves of 12 provenances of *Acacia senegal*. LS = light saturation point, LC = light compensation point, AQE = quantum yield, $A_{max}$ = the assimilation rate at light saturation, $R_d$ = dark respiration and IWUE = instantaneous water use efficiency; n= 8 plants per provenance.

| Provenance | LS (µmol m$^{-2}$) | LC (µmol m$^{-2}$) | AQE | Amax | $R_d$ | IWUE |
|---|---|---|---|---|---|---|
| Bissiga | 2029.33 (456.80) | 13.22 (1.95) | 0.21 (0.06) | 41.37 (9.98) | -2.43 (0.42) | 3.29 (0.18) |
| Maroua | 1531.39 (489.34) | 19.67 (1.70) | 0.15 (0.03) | 37.52 (8.38) | -2.68 (0.26) | 3.46 (0.16) |
| Dara | 1228.18 (175.75) | 17.57 (2.69) | 0.13 (0.02) | 34.09 (5.35) | -2.31 (0.37) | 3.41 (0.18) |
| Fallatu Forest | 1116.04 (188.55) | 14.93 (1.81) | 0.14 (0.01) | 33.73 (5.92) | -1.92 (0.25) | 3.37 (0.15) |
| India ICRAF | 1558.49 (455.77) | 16.65 (3.64) | 0.20 (0.05) | 32.05 (7.68) | -2.19 (0.28) | 3.21 (0.49) |
| Kibwezi | 2509.45 (623.26) | 16.94 (2.62) | 0.17 (0.03) | 43.56 (6.60) | -2.43 (0.30) | 2.83 (0.17) |
| Kiembara | 1559.02 (305.85) | 14.00 (2.27) | 0.17 (0.04) | 42.73 (10.03) | -2.07 (0.34) | 2.31 (0.13) |
| Kigwe | 1424.68 (347.15) | 19.45 (1.89) | 0.14 (0.02) | 38.45 (11.52) | -2.47 (0.26) | 2.94 (0.21) |
| Kirbou | 1309.33 (282.26) | 12.46 (1.46) | 0.16 (0.03) | 32.68 (5.68) | -1.88 (0.29) | 2.86 (0.22) |
| Mali | 1612.53 (268.79) | 16.14 (2.46) | 0.19 (0.02) | 30.43 (3.77) | -2.51 (0.24) | 3.02 (0.12) |
| Ouagadougou | 2561.98 (813.77) | 14.31 (2.38) | 0.20 (0.04) | 60.25 (13.55) | -2.31 (0.25) | 3.26 (0.20) |
| Wangingombe | 2810.52 (518.97) | 14.74 (1.43) | 0.19 (0.02) | 50.41 (8.44) | -2.60 (0.31) | 3.11 (0.11) |

Table 4: Estimated mean (SE) of leaf area (LA), specific leaf area (SLA), root and shoot biomass and $\delta^{13}C$ for water table glasshouse trial. The instrumental standard deviation for the $\delta^{13}C$ values was 0.2537.* indicates either missing data or unable to calculate. There was a Pearson correlation coefficient of 0.854 between $\delta^{13}C$ and root biomass and 0.627 with shoot biomass ($p<0.05$) in 50 cm pots; Pearson correlation coefficient of 0.64 between survival and root biomass ($P<0.05$) in 30 cm pots.

| Pot Depth (cm) | Provenance | Survival | LA ($mm^2$) | SLA ($mm^2 g^{-1}$) | Root (g) | Shoot (g) | $\delta^{13}C$ |
|---|---|---|---|---|---|---|---|
| 30 | Bissiga | 4 | 11.57 (3.42) | 0.33 (0.03) | 4.02 (2.13) | 3.88 (2.15) | -31.57 |
| | Maroua | 1 | * | * | 0.00 (*)[a] | 0.08 (*) | * |
| | Dara | 7 | 10.65 (1.08) | 0.25 (0.01) | 5.51 (1.27) | 6.02 (1.29) | -31.92 |
| | Fallatu Forest | 3 | 13.59 (2.41) | 0.22 (0.01) | 3.15 (0.84) | 5.01 (0.67) | -32.43 |
| | India ICRAF | 7 | 16.46 (1.97) | 0.26 (0.04) | 4.80 (0.92) | 18.20 (4.38) | -31.85 |
| | Kibwezi | 3 | 24.95 (7.68) | 0.24 (0.03) | 4.22 (1.86) | 8.33 (4.25) | -31.31 |
| | Kiembara | 7 | 12.67 (1.12) | 0.26 (0.02) | 3.21 (0.99) | 3.99 (0.91) | -31.99 |
| | Kigwe | 2 | 17.51 (3.33) | 0.20 (0.03) | 4.66 (0.27) | 22.90 (6.72) | -31.32 |
| | Kirbou | 7 | 11.59 (1.75) | 0.26 (0.02) | 3.68 (0.64) | 4.68 (1.06) | -31.61 |
| | Mali | 6 | 14.61 (3.47) | 0.26 (0.01) | 3.43 (0.79) | 5.38 (1.32) | -31.68 |
| | Ouagadougou | 2 | 7.84 (*) | 0.25 (*) | 0.92 (0.90) | 0.89 (0.86) | -31.35 |
| | Wangingombe | 0 | * | * | * | * | * |
| 50 | Bissiga | 6 | 12.93 (2.43) | 0.30 (0.05) | 11.84 (3.09) | 13.56 (4.09) | -31.06 |
| | Maroua | 0 | * | * | * | * | * |
| | Dara | 4 | 11.58 (1.37) | 0.26 (0.03) | 6.99 (2.48) | 8.67 (2.81) | -32.13 |
| | Fallatu Forest | 6 | 10.65 (1.14) | 0.27 (0.02) | 10.63 (0.88) | 18.23 (2.73) | -31.67 |
| | India ICRAF | 6 | 16.15 (1.09) | 0.27 (0.02) | 13.34 (2.17) | 47.77 (4.27) | -31.15 |
| | Kibwezi | 5 | 13.72 (2.17) | 0.29 (0.02) | 5.19 (1.93) | 9.56 (3.80) | -31.90 |
| | Kiembara | 5 | 12.53 (1.40) | 0.26 (0.01) | 9.55 (4.44) | 10.49 (4.86) | -31.81 |
| | Kigwe | 5 | 10.57 (1.92) | 0.18 (0.00) | 11.48 (0.98) | 42.07 (2.31) | -31.33 |
| | Kirbou | 6 | 11.11 (1.94) | 0.28 (0.02) | 8.02 (1.77) | 9.36 (1.30) | -31.77 |
| | Mali | 6 | 9.93 (1.45) | 0.27 (0.04) | 8.35 (1.99) | 11.34 (2.66) | -31.93 |
| | Ouagadougou | 1 | 9.90 (*) | 0.24 (*) | 5.26 (*) | 5.51 (*) | -32.25 |
| | Wangingombe | 1 | 10.30 (*) | 0.37 (*) | 3.66 (*) | 6.13 (*) | -32.85 |

[a] Root too small to determine biomass.

**Figure Captions**

Figure: 1 (a) *Acacia senegal* provenance locations used in the trials in Bambey and Dahra, Senegal (dark circles) and glasshouse in Edinburgh, UK (grey circles). (b) Map of Senegal with administrative regions and locations of the trials.

Figure 2: (a) Mean $\delta^{13}C$ values (‰) in relation to season [please note that although we use the terminology 'dry season' here October is not really the dry season *per se* but the **beginning** of the dry season], site and provenance, sampled at Dahra (wet August and dry October 2009) and Bambey (August only). Error bars represent 95% confidence intervals. (b) Mean $\delta^{13}C$ values in relation to season and life form, error bars represent 95% confidence intervals at Dahra only. Life-forms are as follows: leguminous trees (*Acacia*) non-leguminous trees (trees), shrubs and herbaceous plants (herbs). (c) Correlation between mean $\delta^{13}C$ values in 2009 and mean gum production in 2001, 2007-2009. Linear line is for reference only; Pearson correlation coefficient = - 0.697, p value < 0.05.

Figure 3: (a) Mean $\delta^{15}N$ values (‰) in relation to season [please note that although we use the terminology 'dry season' here October is not really the dry season *per se* but the **beginning** of the dry season], site and provenance; error bars represent 95% confidence intervals. REFD and REFB refer to non-leguminous reference plants at Dahra and Bambey respectively (i.e. includes all life-forms except *Acacia*) (b) Mean $\delta^{15}N$ values in relation to season and life form at Dahra; error bars represent 95% confidence intervals. Life-forms are as follows: leguminous trees (*Acacia*) non-leguminous trees (trees), shrubs and herbaceous plants (herbs).

Figure 4: $\delta^{13}C$ (‰) for the Kankoussa provenance at Dahra only, mean $\delta^{13}C$ values in relation to season [please note that although we use the terminology 'dry season' here October is not really the dry season *per se* but the **beginning** of the dry season] and individual tree. Five leaves measured per tree, error bars represent 95% confidence intervals of the mean.

Figure 5: (a) Axes 1 and 2 of a principal components analysis on soil and climate data from HWSD and LocClim respectively; $\delta^{13}C$ (‰) data area projected into the ordination diagram passively. The cumulative percentage variance of axes 1 and 2 was 76.0 and 85.2 respectively. Some variables have been omitted from the diagram for clarity retaining those with the highest correlations with axes 1 and 2. (b) Axes 1 and 2 of a principal components analysis on soil and climate data from HWSD and LocClim respectively; $\delta^{15}N$ (‰) data area projected into the ordination diagram passively. The cumulative percentage variance of axes 1 and 2 was 56.1 and 81.7 respectively. Again, some variables have been omitted from the diagram for clarity using the same criteria.

Figure 1

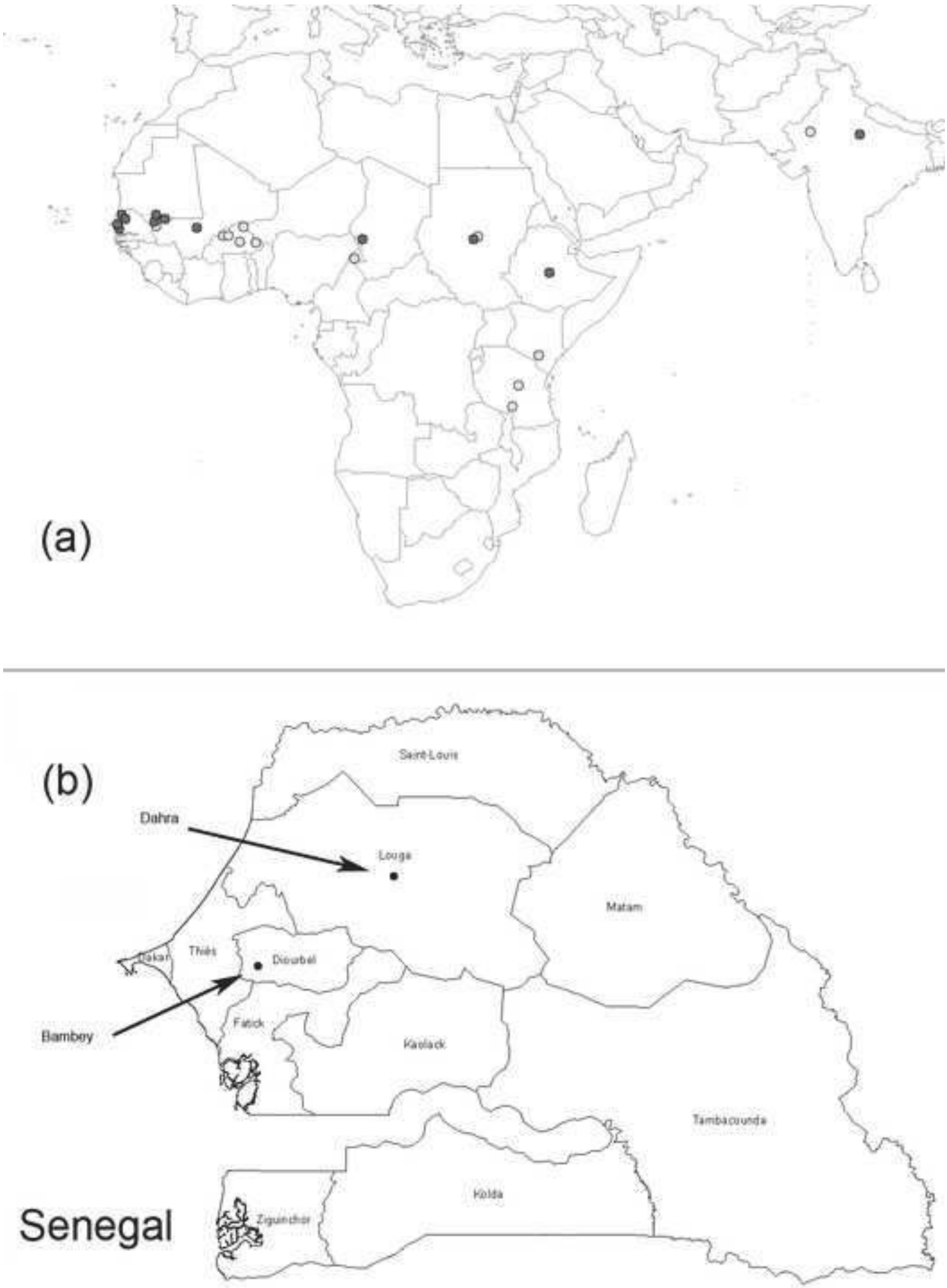
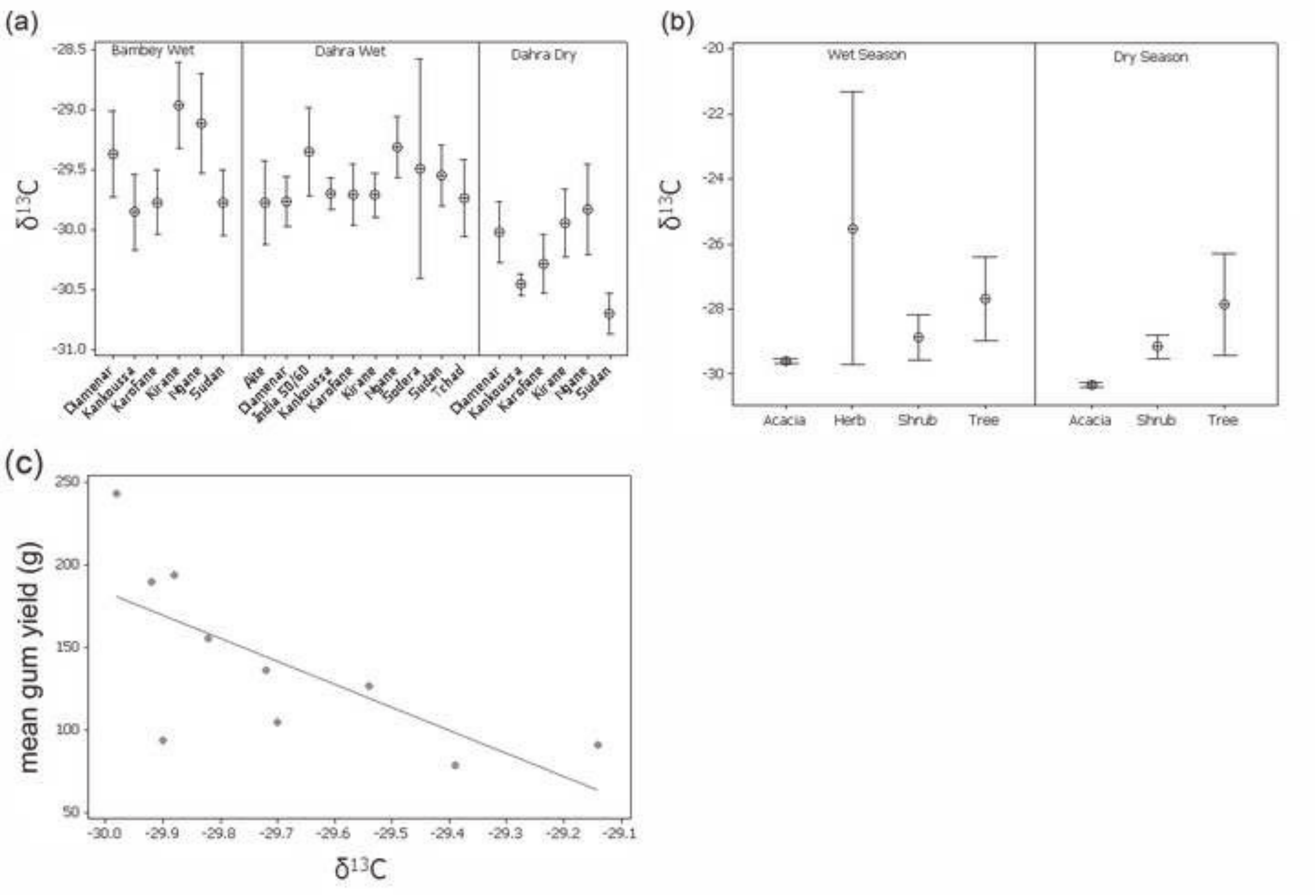
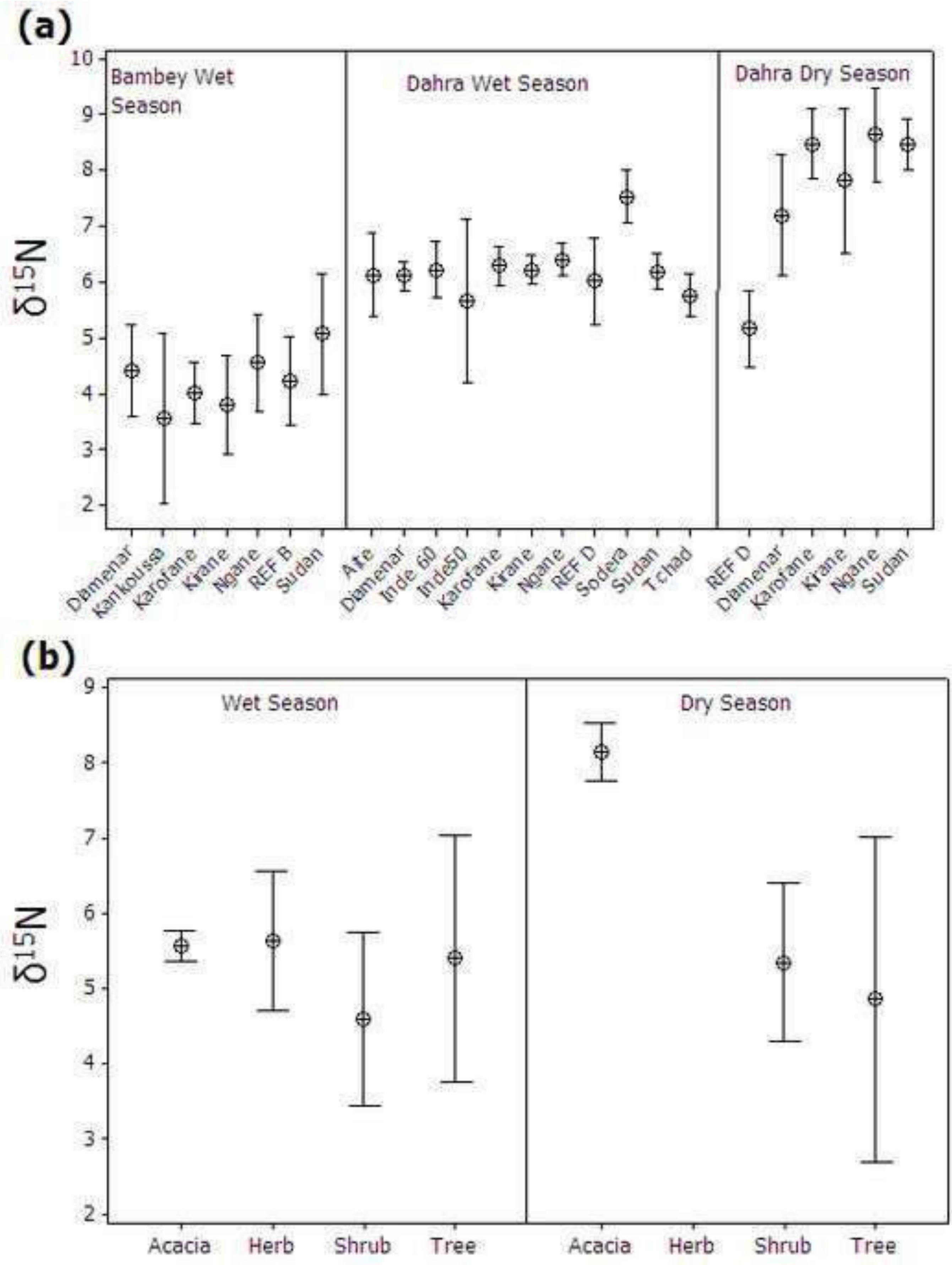

(a)
(b)
Saint-Louis
Dahra
Louga
Matam
Dakar
Thiès
Diourbel
Bambey
Fatick
Kaolack
Tambacounda
Kolda
Ziguinchor
Senegal

Figure 2



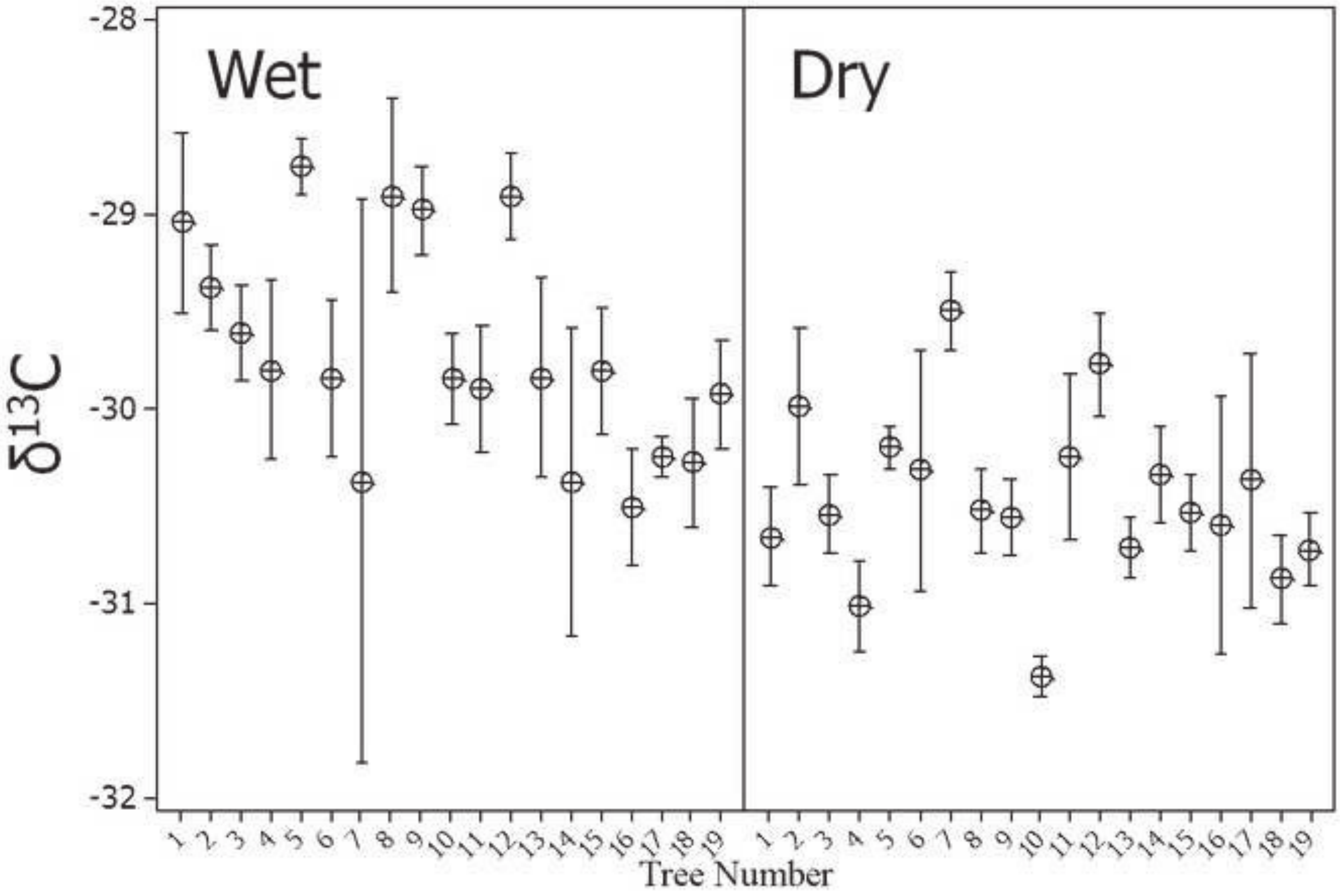
(a)
Bambey Wet
Dahra Wet
Dahra Dry
δ13C
-28.5
-29.0
-29.5
-30.0
-30.5
-31.0
Diamenar
Kankoussa
Karofane
Kirane
Ngane
Sudan
Aite
Diamenar
India 50/60
Kankoussa
Karofane
Kirane
Ngane
Sodera
Sudan
Tchad
Diamenar
Kankoussa
Karofane
Kirane
Ngane
Sudan
(b)
Wet Season
Dry Season
δ13C
-20
-22
-24
-26
-28
-30
Acacia
Herb
Shrub
Tree
Acacia
Shrub
Tree
(c)
mean gum yield (g)
250
200
150
100
50
-30.0
-29.9
-29.8
-29.7
-29.6
-29.5
-29.4
-29.3
-29.2
-29.1
δ13C

Figure 3



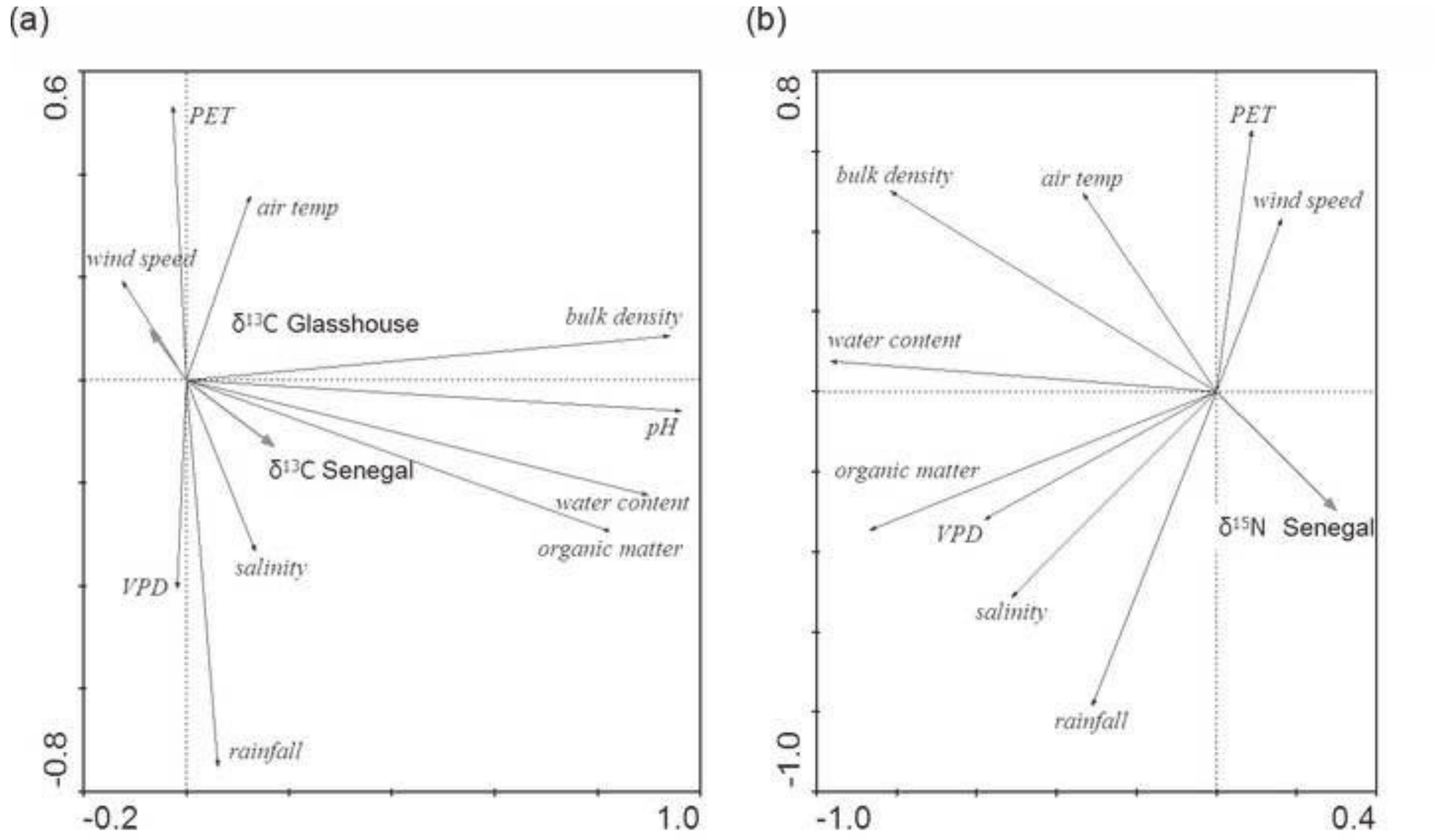
(a)
Bambey Wet Season
Dahra Wet Season
Dahra Dry Season
δ15N
10
9
8
7
6
5
4
3
2
Damenar
Kankoussa
Karofane
Kirane
Ngane
REF B
Sudan
Afre
Damenar
Inde 60
Inde50
Karofane
Kirane
Ngane
REF D
Sodera
Sudan
Tchad
REF D
Damenar
Karofane
Kirane
Ngane
Sudan
(b)
Wet Season
Dry Season
δ15N
9
8
7
6
5
4
3
2
Acacia
Herb
Shrub
Tree
Acacia
Herb
Shrub
Tree

Figure 4



Wet
Dry
δ13C
-28
-29
-30
-31
-32
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Tree Number

Figure 5


(a)
0.6
-0.8
-0.2
1.0
PET
air temp
wind speed
δ13C Glasshouse
bulk density
pH
δ13C Senegal
water content
organic matter
salinity
VPD
rainfall
(b)
0.8
-1.0
-1.0
0.4
PET
bulk density
air temp
wind speed
water content
organic matter
VPD
δ15N Senegal
salinity
rainfall

**Plant and Soil: Supplementary Material**

## Does geographic origin dictate ecological strategies in *Acacia senegal* (L.) Willd.? Evidence from carbon and nitrogen stable isotopes

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Table S1: Mean tree height data for sites and provenances at the Senegal trials.

| Site | Provenance | mean Height (m) | SE |
|---|---|---|---|
| Bambey | Aite | * | * |
| | Diamenar | 2.62 | 0.27 |
| | Kankoussa | 2.93 | 0.27 |
| | Karofane | 2.93 | 0.22 |
| | Kirane | 3.60 | 0.24 |
| | Ngane | 3.31 | 0.25 |
| | Sudan | 3.38 | 0.22 |
| Dahra | Aite | 2.48 | 0.39 |
| | Diamenar | 3.65 | 0.19 |
| | Inde 60 | 2.16 | 0.19 |
| | Inde50 | 2.70 | 0.62 |
| | Karofane | 2.94 | 0.20 |
| | Kirane | 3.85 | 0.17 |
| | Ngane | 3.66 | 0.19 |
| | Sodera | 1.87 | 0.30 |
| | Sudan | 3.84 | 0.19 |
| | Tchad | 2.50 | 0.36 |

Table S2: Environmental variables used in the principal coordinates analyses with abbreviated codes used in Figure 5 and corresponding units.

| Database | Environmental Variable | Figure 5 Code | Units |
|---|---|---|---|
| HWSD | water content | AWC | mm |
| | Topsoil Sand Fraction | | % |
| | Topsoil Silt Fraction | | % |
| | Topsoil Clay Fraction | | % |
| | Topsoil Reference Bulk Density | Top Bulk | kg/dm3 |
| | Topsoil Gravel Content | | % |
| | Topsoil Organic Carbon | Top OM | % weight |
| | Topsoil pH | Top pH | in $H_2O$ |
| | Topsoil CEC (clay) | | cmol/kg |
| | Topsoil CEC (soil) | | cmol/kg |
| | Topsoil Base Saturation | | % |
| | Topsoil TEB | | cmol/kg |
| | Topsoil Calcium Carbonate | | % weight |
| | Topsoil Gypsum | | % weight |
| | Topsoil Sodicity (ESP) | | % |
| | Topsoil Salinity (ECe) | Top Sal | dS/m |
| | Subsoil Sand Fraction | | % |
| | Subsoil Silt Fraction | | % |
| | Subsoil Clay Fraction | | % |
| | Subsoil Reference Bulk Density | Sub Bulk | kg/dm3 |
| | Subsoil Gravel Content | | % |
| | Subsoil Organic Carbon | | % weight |
| | Subsoil pH | | in $H_2O$ |
| | Subsoil CEC (clay) | | cmol/kg |
| | Subsoil CEC (soil) | | cmol/kg |
| | Subsoil Base Saturation | | % |
| | Subsoil TEB | | cmol/kg |
| | Subsoil Calcium Carbonate | | % weight |
| | Subsoil Gypsum | | % weight |
| | Subsoil Sodicity (ESP) | | % |
| | Subsoil Salinity (ECe) | | dS/m |
| LocClim | Potential evapotranspiration | PET | mm |
| | Annual rainfall | Rain | mm |
| | Sunshine hours | | hrs |
| | Mean temperature | T mean | $^oC$ |
| | Maximum temperature | | $^oC$ |
| | Minimum temperature | | $^oC$ |
| | Vapour pressure deficit | VPD | kPa |
| | Standard deviation of rainfall | | mm |
| | Wind speed | Wind | km hr-1 |

Table S3: Provenance variation in BNF estimates of *Acacia senegal* provenances evaluated on foliar samples from Bambey and Dahra sites beginning (wet season, August 2009) of rains. Means (±standard error, SE) in leaf N (%) and the proportion of foliar N derived from BNF (% Ndfa). Estimates were based on *Balanites aegyptiaca* reference plants ($\delta^{15}N_{ref}$) values: Bambey site, 7.32 ‰ ($n$ = 1); Dahra site, 6.77 ± 0.50 ‰ ($n$ = 3).

| **Site** | ***n*** | **Provenance** | **% N± SE** | **$\delta^{15}$N(‰)± SE** | **% Ndfa± SE** |
|---|---|---|---|---|---|
| Bambey | 12 | Kirane, Mali | 3.40±0.15 | 3.81 ± 0.40 | 35.7 ± 4.1 |
| | 15 | Karofane, Niger | 3.98±0.10 | 4.03 ± 0.26 | 33.5 ± 2.6 |
| | 11 | Diamenar, Senegal | 4.05±0.14 | 4.40 ± 0.37 | 29.7 ± 3.8 |
| | 7 | Ngane, Senegal | 3.38±0.17 | 4.55 ± 0.36 | 28.1 ± 3.7 |
| | 15 | Kordofan, Sudan | 3.85±0.12 | 5.07 ± 0.51 | 22.9 ± 5.2 |
| | 6 | Kankoussa, Mauritania | 4.26±0.28 | 3.56 ± 0.60 | 38.2 ± 6.1 |
| Dahra | 18 | Kirane, Mali | 4.36±0.09 | 6.22 ± 0.12 | 5.9 ± 1.3 |
| | 10 | Aite, Mali | 4.58±0.07 | 6.13 ± 0.33 | 6.9 ± 3.6 |
| | 17 | Karofane, Niger | 4.48±0.09 | 6.30 ± 0.16 | 5.1 ± 1.7 |
| | 20 | Diamenar, Senegal | 4.63±0.11 | 6.11 ± 0.12 | 7.1 ± 1.3 |
| | 18 | Ngane, Senegal | 4.20±0.11 | 6.41 ± 0.12 | 3.9 ± 1.3 |
| | 20 | Kordofan, Sudan | 4.38±0.11 | 6.19 ± 0.16 | 6.3 ± 1.7 |
| | 6 | Sodera, Ethiopia | 3.36±0.08 | 7.54 ± 0.19 | -0.3 ± 2.0 |
| | 8 | Jodhpur Inde60, India | 4.20±0.15 | 6.22 ± 0.21 | 5.9 ± 2.3 |
| | 5 | Jodhpur Inde50, India | 4.45±0.27 | 5.66 ± 0.53 | 11.9 ± 5.7 |
| | 9 | Tourba, Chad | 4.72±0.15 | 5.77 ± 0.17 | 10.8 ± 1.8 |

Table S4: Unbalanced ANOVA table for photosynthetic parameters, light saturation (top) and Amax (below).

| Factor | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| + Block | 7 | 25653685. | 3664812. | 2.75 | 0.013 |
| + Provenance | 11 | 30692703. | 2790246. | 2.10 | 0.031 |
| Residual | 74 | 98546161. | 1331705. | | |
| Total | 92 | 154892548. | 1683615. | | |
| Factor | d.f. | s.s. | m.s. | v.r. | F pr. |
| + Block | 7 | 7768.7 | 1109.8 | 2.61 | 0.018 |
| + Provenance | 11 | 7981.7 | 725.6 | 1.71 | 0.088 |
| Residual | 74 | 31445.4 | 424.9 | | |
| Total | 92 | 47195.8 | 513.0 | | |

Figure S1: *Acacia senegal* provenance trial schematic for the Dahra Station, Provenances that were sampled for $^{13}C$ and $^{15}N$are highlighted in yellow, note that in each provenance plot there were 25 trees randomly planted for each provenance. Also note that there was a 5 m buffer strip between blocks.

| **Block 4** | | **Block 3** | | **Block 2** | | **Block 1** | |
|---|---|---|---|---|---|---|---|
| Sodera | Djigueri | Inde 60 | Ngane | Kirane | Sodera | Kirane | Sodera |
| Daiba | Kidira | Soudan | Kidira | Diamenar | Djigueri | Karofane | Aite |
| Burkina DI | Inde 60 | Diamenar | Daiba | Kankoussa | Karofane | Soudan | Kankoussa |
| Diamenar | Ngane | Aite | Inde 50 | Somo | Soudan | Djigueri | Diamenar |
| Kankoussa | Aite | Bissiga | Tchad | Aite | Pakistan | Pakistan | Somo |
| Somo | Inde 50 | Sodera | Karofane | Kidira | Tchad | Burkina DI | Ngane |
| Tchad | Karofane | Djigueri | Kirane | Daiba | Burkina DI | Bissiga | Inde 60 |
| Bissiga | Soudan | Kankoussa | Somo | Ngane | Inde 50 | Tchad | Kidira |
| Pakistan | Kirane | Pakistan | Burkina DI | Inde 60 | Bissiga | Inde 50 | Daiba |

Figure S2: $\delta^{13}C$ values for leaves of *Rumex acetosa* L. dried in microwave oven and conventional oven; error bars represent instrumental SD.