

Interaction between grapevines and trees: effects on water relations, nitrogen nutrition, and wine

Carina P. Lang^a, Nikolaus Merkt^a, Christoph-Martin Geilfus^b, Simone Graeff-Hönninger^c, Judy Simon^d, Heinz Rennenberg^e and Christian Zörb^a

^aInstitute of Crop Sciences, Quality of Plant Products, University of Hohenheim, Stuttgart, Germany;

^bAlbrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Controlled Environment

Horticulture, Humboldt-University of Berlin, Berlin, Germany; ^cInstitute of Crop Sciences, Agronomy,

University of Hohenheim, Stuttgart, Germany; ^dDepartment of Biology, Plant Interactions Ecophysiology

Group, University of Konstanz, Konstanz, Germany; ^eInstitute of Forest Sciences, Chair of Tree Physiology,

University of Freiburg, Freiburg, Germany

ABSTRACT

Agroforestry systems (AF) consisting of grapevines and trees, may lead to resource competition for water and nutrients. This study aimed to evaluate the impact of a combined cultivation on water relations, nitrogen nutrition and the resulting wine quality.

¹⁵N-labeled inorganic nitrogen (N) sources were used to quantify net N uptake capacity. N content and $\delta^{15}\text{N}$ natural abundance were analysed as integrating parameters of N nutrition. Leaf water potential (ψ_{leaf}) was determined to evaluate the water status of grapevines. Wine quality was evaluated by chemical and sensory analyses. In result, AF system reduced leaf water potential and increased net N uptake capacity in grapevines. However, chemical composition and sensory quality of the wine were not significantly affected in the present system consisting of Riesling, Sauvignon Blanc, oak and poplar.

Nitrogen availability of grapevines was favourable and water relations were improved, whereas wine quality was similar when grown with trees or without. Trees were able to reduce water and nitrogen losses without negative effects on wine quality.

This work provides information on benefits and limits for intercropping of trees and grapevines in terms of performance of grapevines and wine quality compared to traditional vineyard systems.

KEYWORDS

Agroforestry; grapevine; nitrogen; water; wine

Introduction

Agroforestry systems (AF) are land-use systems that combine woody perennials with agricultural crops, animals or both on the same unit of land (Lundgren and Raintree 1983). In the present study, we focused on an agri-silvicultural system, consisting of vines as a woody perennial crop and trees, which was traditionally used in southern Europe, such as Italy, Portugal, Spain, Greece and France; and was called *Piantata* or *Vitis arbusta* in Italy, or *Joualle* in France (Altieri and Nicholls 2002; Eichhorn et al. 2006; Nerlich et al. 2013). The combined cultivation of vine with woody perennials gives rise to interspecific interactions, being either competitive or synergetic. For example, trees have the potential to build a physical barrier for weeds and insects, alter microclimate, raise

CONTACT Christian Zörb  Christian.zoerb@uni-hohenheim.de  Institute of Crop Science, Quality of Plant Products, University of Hohenheim, Stuttgart, Germany

biodiversity, enhance soil fertility or even improve air and water quality (Jose 2009). On the other hand, trees may act as competitors for resources such as light, space, nutrients and/or water (Jose et al. 2004; Bainard et al. 2011), and it was shown that especially water and nitrogen (N) availability are strongly linked to each other (Hu et al. 2013). Nitrogen is an important growth-promoting nutrient for trees (Rennenberg and Dannenmann 2015). In vine, N availability influences not only yield and growth, but is of utmost importance for the concentration of amino acids and N-containing secondary metabolites in berries that are also relevant for the wine quality. Furthermore, yeast-assimilable nitrogen (YAN) is important for the fermentation of the must because it influences yeast growth and fermentation kinetics (Bell and Henschke 2005). Aroma compounds do not only arise from must, but also originate from products of yeast metabolism, especially from sugar and N compounds present in the grapes (Mendes-Ferreira et al. 2011). Water supply strongly determines vine phenology and grape ripening (Van Leeuwen et al. 2009). A shortage in water supply may have adverse effects on the development of grapevines and quality formation of the wine (Keller 2005; Chaves et al. 2010; Lovisolo et al. 2010). On the other hand, a moderate lack of water may lead to the adaptive accumulation of metabolites such as phenols or anthocyanins, favourable for vine quality and sensory features (Ribéreau-Gayon et al. 2006; Deluc et al. 2009; Lovisolo et al. 2016). Nitrogen nutrition and water availability are interlinked, because water acts as a solvent for N compounds in the soil, facilitating uptake from the soil solution into the root. However, it is not known whether a competition between vines and trees can lead to impaired water and N nutrition in AF systems, especially if the tree species has high in water demand, and whether this results in quality changes of the vines and the resulting wine.

In the present study we investigated whether an agri-silvicultural AF system, consisting of vine (*Vitis vinifera* L. cv. Riesling or cv. Sauvignon Blanc) and poplar (*Populus alba* or *Populus tremula* x. *P. alba*) or oak (*Quercus petraea*) trees, was associated with impaired water relations and N nutrition of the grapevines, and if this AF system altered the quality of the wine. These two tree species were chosen because they greatly differ in terms of resource needs, e.g. oak has low, and poplar has high water- and N requirements. To this aim we used $\delta^{15}\text{N}$ -labelled organic and inorganic N sources to quantify net N uptake capacity (nNUC), while leaf water potential (ψ_{leaf}) and $\delta^{13}\text{C}$ abundance were determined to evaluate the water status of the vines. Moreover, the wine quality in terms of sugar, phenols and quality-determining acids was measured and the sensory profile as well as flavour and odour were evaluated. With this experiment we evaluated whether the water and N supply to the vines, as well as wine quality, was more affected in comparison of the AF system with poplar or oak.

Material and methods

Plant material and experimental conditions

The field experiment was conducted in 2013 and 2015 in a 0.50-ha experimental vineyard in Ayl, Rhineland-Palatinate, Germany (Long. 49°37'N, Lat. 006°32'E), and consisted of grapevines and trees grown in an agroforestry (AF) system. The AF system was established in 2007 when oaks were three years old and poplars were one year old. The soil is classified as a horticultural anthrosol with a skeleton fraction of 20–30% and 15% clay. Grapevines *Vitis vinifera* L. cv. Riesling (R) and *Vitis vinifera* L. cv. Sauvignon Blanc (S) (both grafted on rootstock Selection Oppenheim 4 (SO4)) in a wine nursery and one year old at planting were arranged as monoculture (control group), and as a mixed cropping system with oak (*Quercus petraea*) (RO, SO) or poplar (*Populus tremula* x. *P. alba*) (RP, SP). In addition trees were also planted as monoculture (O, P) as controls. Imperfections (population losses based on accretion problems) in the existing tree population of *P. tremula* x. *P. alba* were filled with trees of *P. alba*. Trees were pruned periodically to a height of 3 m. In total the vineyard was divided into 36 plots (12 m × 10 m; see supplemental data, S.1). Treatments included monocultures of each species (15 trees and 25 vines per plot, respectively, four replicates each), and combinations of vines and trees in every variation (mixed cropping systems, five replicates

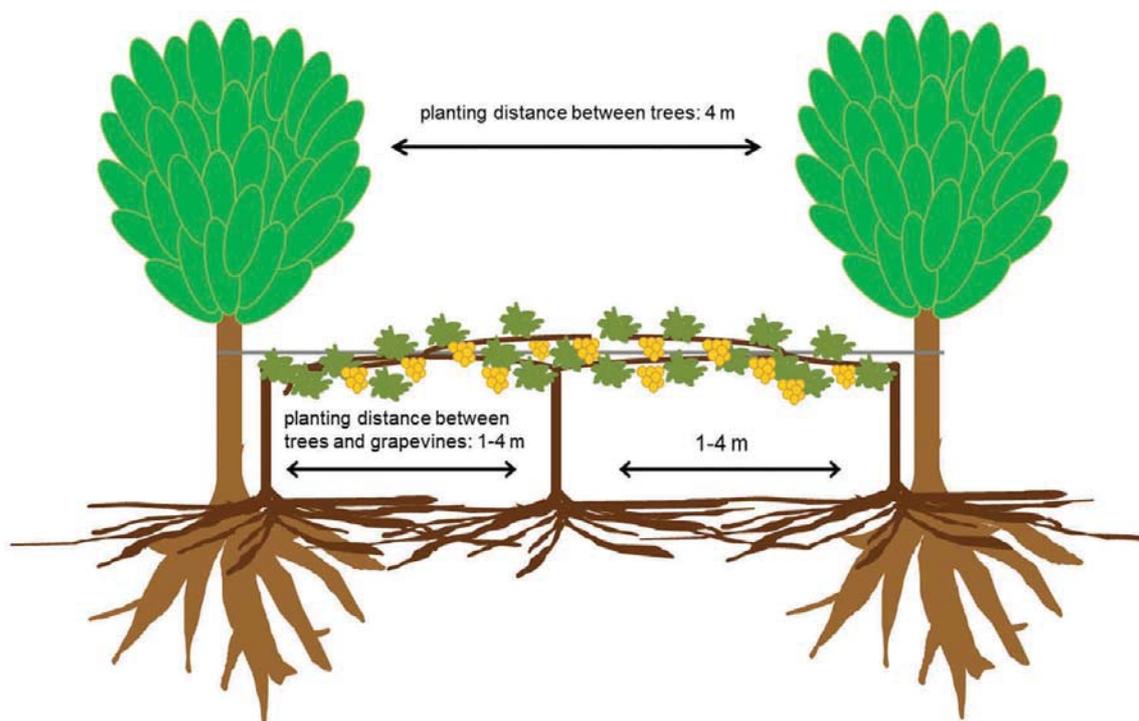


Figure 1. Schematic view of the cultivated agroforestry system. Section of a vine row and trees. The planting distance between trees is 4 m. The planting distance between trees and vines is 1-4m, depending on cultivation as monoculture or mixed cropping system.

each). The set-up was a fully randomized experimental block design with an inclination of 26.6%. Rows were planted in a SE/ESE direction with spacing of 2 m. The spacing between trees and vines among one row was 4 m and the spacing within and depending on the necessary space for tree and vine growth (Figure 1). Annual precipitation [mm], average temperature [°C] and sunshine duration [h] from 2013 and 2015 are given in the supplemental data (S.2). Data were taken from the nearest official weather station 'Trier Petrisberg' (Long. 49°45'N, Lat. 006°40'E), of the German Meteorological Service.

Leaf water potential measurements

Leaf water potential (ψ_{leaf}) of grapevines was measured in September 2013, at BBCH 85 –89, using a Scholander pressure chamber (Scholander et al. 1965). The measurements took place pre-dawn. The date was chosen, because at this stage of development, berries started to soften and had a high water requirement. In every plot, four fully expanded vines were randomly chosen. From these vines always the youngest fully expanded apical leaf were sampled. Values are expressed in MPa.

Determination of nitrate, ammonium, arginine and glutamine net uptake capacity in grapevine roots

N net uptake capacity was determined by ^{15}N -tracer labelling experiments as previously described for beech (*Fagus sylvatica*) and spruce (*Picea abies*) (Gessler et al. 1998). Since grapevines have a cyclical N demand and uptake with a maximum between bloom and pea-size (June-July) (Hanson and Howell 1995), uptake studies were conducted in July 2015. Samples of grapevine fine root (five fine roots per plant, located in the upper 5–10 cm of the soil) were taken from all cropping systems between 10 am and 2 pm to avoid diurnal variation (Gessler et al. 2002). Six biological replicates [$n = 6$] were analysed per plot. The fine roots were carefully dug free from soil, by using a small scraper and a brush. Coarse dirt was removed and intact roots were incubated for

2 h in an artificial soil solution with the following nutrient composition: 100 μM KNO_3 , 1 μM NH_4Cl , 25 μM Gln (glutamine), 10 μM Arg (arginine), 10 μM AlCl_3 , 90 μM CaCl_2 , 7 μM FeSO_4 , 50 μM KCl , 6 μM K_2HPO_4 , 24 μM MnCl_2 , 20 μM NaCl and 70 μM MgCl_2 . Five nutrient solutions containing different labelled N sources were used: ammonium ($^{15}\text{NH}_4^+$) or nitrate ($^{15}\text{NO}_3^-$) as inorganic N forms, or glutamine ($^{15}\text{N}^{13}\text{C}$ -Gln) or arginine ($^{15}\text{N}^{13}\text{C}$ -Arg) as organic N forms. The fifth solution was used as control and did not contain labelled N. After incubation, the roots were cut off from the vines and washed twice with 0.5 M CaCl_2 solution to remove adhering nutrients and carefully blotted dry. For later analyses of ^{15}N , roots were dried for two days at 60°C and ground at 25.5 s^{-1} for 45 seconds using a vibrating tube mill (MM 301, Retsch, Haan, Germany). Fresh and dry weights were documented. Net N uptake capacities ($\text{nmol N g}^{-1}\text{ fw h}^{-1}$) were calculated from the incorporation of ^{15}N into the root material according to the equation published by Kreuzwieser et al. (2002):

$$\text{Net N uptake} = ((^{15}\text{N}_i - ^{15}\text{N}_n) \times \text{N}_{\text{tot}} \times \text{dw} \times 105) / (\text{MW} \times \text{fw} \times t),$$

where $^{15}\text{N}_i$ and $^{15}\text{N}_n$ are the atom% of ^{15}N in labelled (N_i , labelled) and non-labelled (N_n , natural abundance) roots, respectively; N_{tot} is the total N percentage, MW the molecular weight of ^{15}N , dw is dry weight and t is the time of exposure.

Leaf sampling

In July 2015 leaf samples were collected in all 36 plots, from two randomly selected vines as well as from two randomly selected trees. Leaves were chosen based on age, habitus and diseases, in order to collect uniform sample material. For each vine, the tenth apical leaf was harvested from a healthy shoot (Alleweldt et al. 1982). For consistent sampling of the trees, the fifth leaf of two individual second order branches were harvested. These leaves were chosen because they represent fully developed leaves with the highest rate of photosynthesis. The samples were frozen in dry ice, ground to a fine powder in liquid N_2 , and stored at -80°C until further analyses. For element and stable isotope analyses, aliquots of the powder were dried for two days at 60°C .

Element of stable isotope analyses of C and N in leaf and root tissues

Total carbon (C) and N concentrations as well as ^{13}C and ^{15}N abundance were determined in oven-dried, finely ground leaf (1.4–2.0 mg) and root (1.5–2.2 mg) material using an elemental analyser (NC 2500, CE Instrument, Milan, Italy) coupled via a ConFlo II Interface to an isotope-ratio mass spectrometer (Finnigan MAT GmbH, Bremen, Germany). A working standard (glutamic acid) was calibrated against the primary standards of the U.S. Geological Survey USGS 40 and USGS 41 for quantification of $\delta^{13}\text{C}$ abundance and USGS 25 and USGS 41 for quantification of $\delta^{15}\text{N}$ abundance (Qi et al. 2003; Coplen et al. 2006). The working standard was analysed after every 10th sample to account for potential instrument drift over time as reported by Simon et al. (2011).

Wine samples and processing

Grapes were hand-harvested on October 1st (Sauvignon Blanc) and on October 22th (Riesling) 2013, respectively, when the local defined must weight was reached. All grape bunches were harvested from each plot, but due to technical limitations, only one wine was produced from the four replicate plots of each cropping system. Vinification was done at the 'Dept. of Quality of Plant Products', Institute of Crop Science at the University of Hohenheim, Stuttgart, Germany. A total yield of between 9 L and 14 L of must was collected from each cultivation system by squeezing the berries using a hydraulic press. After pressing, 2 g L^{-1} bentonite was added. After 24 h of cooling at 2°C in a cold store, musts were separated from trub and enriched with 20 g L^{-1}

sucrose. Thiamine and the wine yeast nutrient 'NutriVin' (Anchor, Johannesburg, South Africa) were added. The musts were inoculated with 0.3 g L^{-1} yeast (Anchor Vin 2000; *S. cerevisiae*) and rested till the end of fermentation, when wines were separated from the sedimented yeast and sulphured with 200 mg L^{-1} potassium disulphide ($\text{K}_2\text{S}_2\text{O}_5$). While Sauvignon Blanc wines showed a satisfactory natural purification during sedimentation of the trub, Riesling samples had to be filtered before filling in bottles. Wines were stored in bottles in the wine cellar (12°C ambient temperature) of the University of Hohenheim for 1.5 years before tasting. Sauvignon Blanc was not available in 2015.

Wine analyses

Wine analyses of the vintage 2013 were conducted after the vinification process. pH and total acids (Schmitt 1983) were measured with a titrator (TitroLine easy, Schott, Mainz). Phenolics were determined spectrophotometrically using the Folin-Ciocalteu reagent according to Singleton et al. (1999). Sugars, mainly fructose and glucose, lactic acid, tartaric acid and malic acid were analysed by high performance liquid chromatography (HPLC), (Merck-Hitachi, Darmstadt, Germany). The determination of the different sugars by HPLC is based on Mast et al. (2015). For the determination of the different acids, sulphuric acid (50 mM) was used as the mobile phase with a flow rate of 0.5 ml min^{-1} . Detection was made at 210nm . Phenomenex SecurityGuard Cartridges, Carbo-H $4 \times 3.0\text{mm}$ as precolumn and Phenomenex Rezex™ ROA-Organic Acid H^+ (8%), LC Column $300 \times 7.8 \text{ mm}$, Ea as separation column were used.

Wine sensory analysis

We were able to conduct descriptive sensory analysis of the wine Riesling and Sauvignon Blanc only of 2013 using a trained tasting panel consisting of 10–12 persons. The technical repetition of the wine samples at another day is necessary to account for daily variation in the sensory perception of each panel member. The six wines were tested for intensity in two replications at random order and ambient temperature. For evaluation of the Sauvignon Blanc variations, the panellists were given a total of nine defined attributes, seven for aroma (cassis, green pepper, green grass, passion fruit, asparagus, gooseberry and lemon) and two for flavour/odour (intensity; high/low). Twelve attributes, ten for aroma (pineapple, apple, pear, cassis, petrol, honey, mint, peach, rose and lemon) and two for flavour/odour (intensity; high/low) were used for evaluation of the Riesling variations. For scoring an established four – point scale was provided, with 0 for non-characteristic intensity and 4 for high/extreme intensity.

Statistical analysis

Statistical tests of the data were performed using SAS software (version 9.4, Cary, North Carolina, U.S.A.). A MIXED MODEL, a Kenward-Roger-test with a correction after Tukey-Kramer ($p \leq 0.05$) was used. Studied factors were N source and cropping system. All chemical attributes were analysed separately, and pH values were log-transformed before analysis. For sensory analyses, each aroma attribute was separately analysed and compared between the different wine samples. Sauvignon Blanc and Riesling monocultures served as control for their respective AF systems.

Principal component analysis (PCA) was carried out by using the program XLSTAT (<https://www.xlstat.com/de/>).

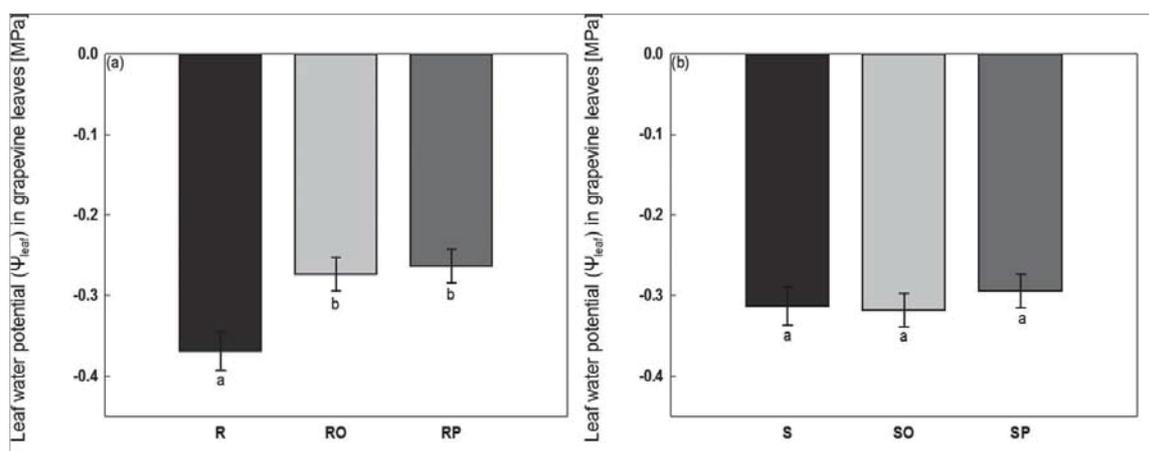


Figure 2. Leaf water potential (Ψ_{leaf}) [MPa] in grapevine leaves of the six different cultivation systems (a) Riesling (R; ■); Riesling/oak (RO; ▒); Riesling/poplar (RP; ▓) and (b) Sauvignon Blanc (S; ■); Sauvignon Blanc/oak (SO; ▒); Sauvignon Blanc/poplar (SP; ▓). Bars represent means \pm SE (single cropping $n = 16$, mixed cropping $n = 20$). Cultivation systems of Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Different letters indicate significant differences; MIXED MODELS, $p \leq 0.05$.

Results

Leaf water potential ψ_{leaf} of grapevine in dependency to the AF

Vitis vinifera L. cv. Riesling and cv. Sauvignon Blanc had different leaf water potential (Ψ_{leaf}) when grown in the studied AF system (mean R = -0.30 ; S = -0.31) Figure 2. Leaf water potential of Riesling was significantly reduced in the AF system by 26.0% (RO) and 28.7% (RP), respectively, compared to the monoculture (Figure 2(a)), while there was no significant effect of the different AF for Sauvignon Blanc (Figure 2(b)).

Total leaf N- and C-concentration and C:N ratio

The total leaf N concentration was increased in both varieties when grown together with oak, RO raised by 22.5% and SO raised by 23.4%, compared to the monoculture (Figures 3(a) and 3(b)). No significant differences were found for total C concentration comparing the AF systems of Riesling (Figure 3(c)) and Sauvignon Blanc (Figure 3(d)). Despite the observed increase in total leaf N concentrations, no significant effects were observed for C:N ratios in both grapevine varieties (R Fig. 3e and S Fig. 3f)

Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$

The isotopic signatures in the discrimination of $\delta^{13}C$ have no significant differences, neither in a monoculture (mean $\delta^{13}C_{\text{‰}}$ R; S: = -26.23), nor in an AF system (mean $\delta^{13}C_{\text{‰}}$ RO; RP; SO; SP: = -26.63) (Figure. 4a and 4b).

The $\delta^{15}N$ abundances were significantly higher compared to atmospheric N (mean $\delta^{15}N_{\text{‰}}$ = zero) for both Riesling (mean $\delta^{15}N_{\text{‰}}$ = 2.403) and Sauvignon Blanc (mean $\delta^{15}N_{\text{‰}}$ = 1.270) monocultures (Figures 4(c) and 4(d)). They were reduced in all AF systems, but the extent of the reduction was higher when grown with oak compared to poplar (mean $\delta^{15}N_{\text{‰}}$ RO: = 0.079; RP: = 0.818; SO: = 0.290; SP: = 0.713).

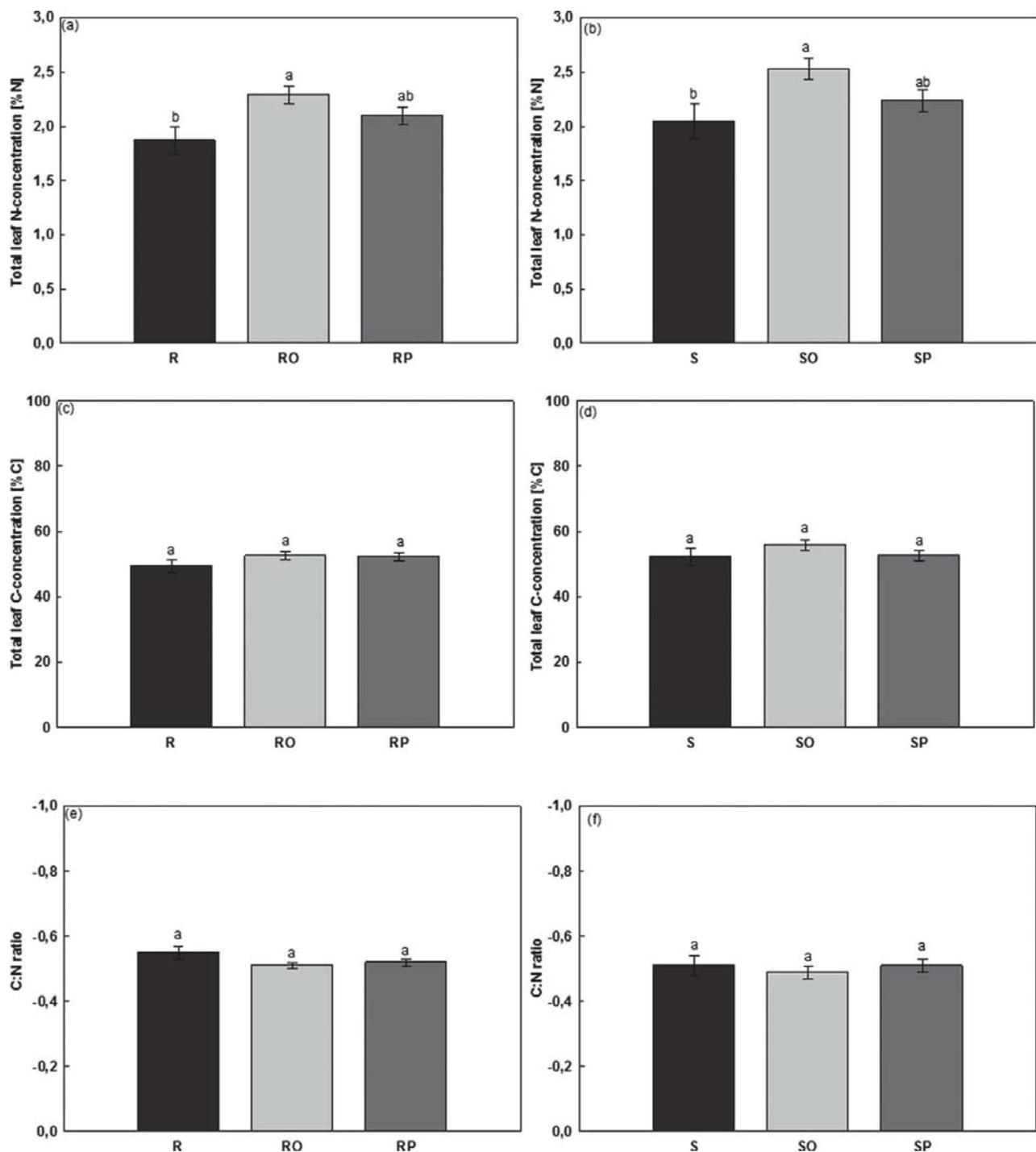


Figure 3. Total N-concentration [%N], total C-concentration [%C] in leaves of the six different cultivation systems (a) & (b) [%N] and (c) & (d) [%C] and C:N ratio in leaves (e) & (f); R (■); RO (□); RP (▒) and S (■); SO (□); SP (▒). Bars represents means ± SE (single cropping n = 8, mixed cropping n = 20). Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Different letters indicate significant differences; MIXED MODELS, $p \leq 0.05$.

Net uptake capacity in fine roots of different nitrogen forms

Across all AF systems, Riesling had significantly higher nNUC for NO_3^- (mean $16.5 \text{ nmol N g}^{-1} \text{ fw h}^{-1}$) compared to NH_4^+ (mean $6.4 \text{ nmol N g}^{-1} \text{ fw h}^{-1}$). Even though the effects of the cropping systems (R; RO; RP) were not statistically significant, there was a tendency for the cultivation system RP (mean $16.6 \text{ nmol N g}^{-1} \text{ fw h}^{-1}$) to have the highest net uptake capacity for both NO_3^- and NH_4^+ (Figure 5(a)).

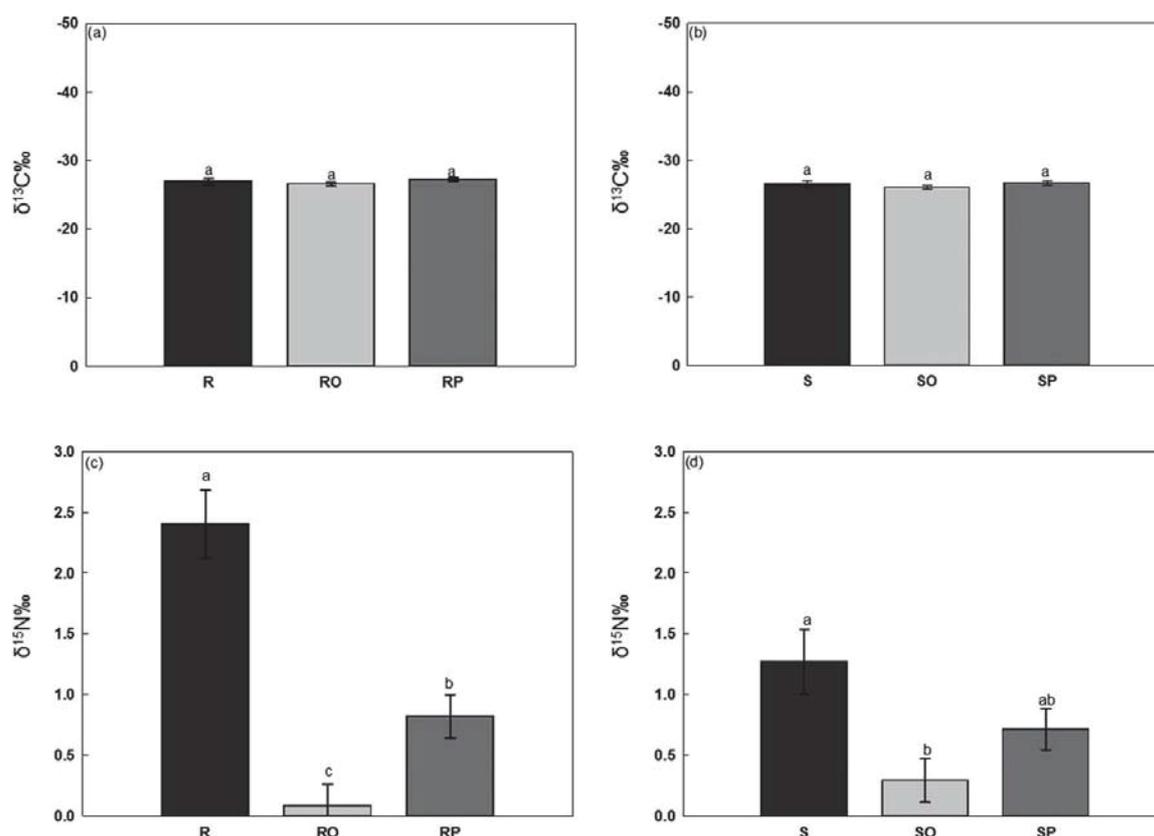


Figure 4. Carbon (a) & (b) isotope composition [$\delta^{13}\text{C}\text{‰}$] in and nitrogen (c) & (d) isotope composition [$\delta^{15}\text{N}\text{‰}$] in leaves of the six different cultivation systems (a) & (c) R (■); RO (▒); RP (■) and (b) & (d) S (■); SO (▒); SP (■). Bars represent means \pm SE (single cropping $n = 8$, mixed cropping $n = 20$). Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Different letters indicate significant differences; MIXED MODELS, $p \leq 0.05$. Exposition period: 2 h.

For Sauvignon Blanc, the nNUC across all systems (S; SO; SP) for NO_3^- (mean $14.5 \text{ nmol N g}^{-1} \text{ fw h}^{-1}$) was slightly, but not significantly, higher than that for NH_4^+ (mean $7.5 \text{ nmol N g}^{-1} \text{ fw h}^{-1}$). The highest nNUC values were observed in SO, with a significantly higher nNUC for NO_3^- , compared to NH_4^+ (Figure 5(b)).

Regarding organic N forms, mean nNUC values across all systems were higher for Arg (214.1 (R) and 145.9 (S) $\text{nmol N g}^{-1} \text{ fw h}^{-1}$) compared to Gln (61.4 (R) and 48.3 (S) $\text{nmol N g}^{-1} \text{ fw h}^{-1}$) for both varieties (Figures 5(c) and 5(d)). A significant difference between the cropping system was only seen for Riesling where nNUC for Arg was higher in the monoculture compared to the RO mixed cropping system.

Wine quality

Only few significant changes in wine composition were detected for the different AF systems (Table I). Compared to the Riesling monoculture (R), total acid concentration was increased in RO (R vs. RO), and lactic acid concentration and sugars decreased in RP (R vs. RP). For Sauvignon Blanc, the combination with oak (SO) resulted in lower pH values and increased sugar concentrations (S vs. SO), while total acid concentration was reduced in SP (S vs. SP) (Table 1). A principal component analysis (PCA) Biplot provides a visualization of the two principal components by identifying groups (Ringnér 2008). In the present study, Riesling and its AF clustered away from Sauvignon Blanc and its AF (Figure 6). The separation was based on the loadings of the second PC. Furthermore, oak had the highest impact (longest cluster distance from the respective monoculture) on the chemical composition of both wine varieties. Overall, the PCA indicated that the changes of the chemical attributes of the Riesling wines were mainly

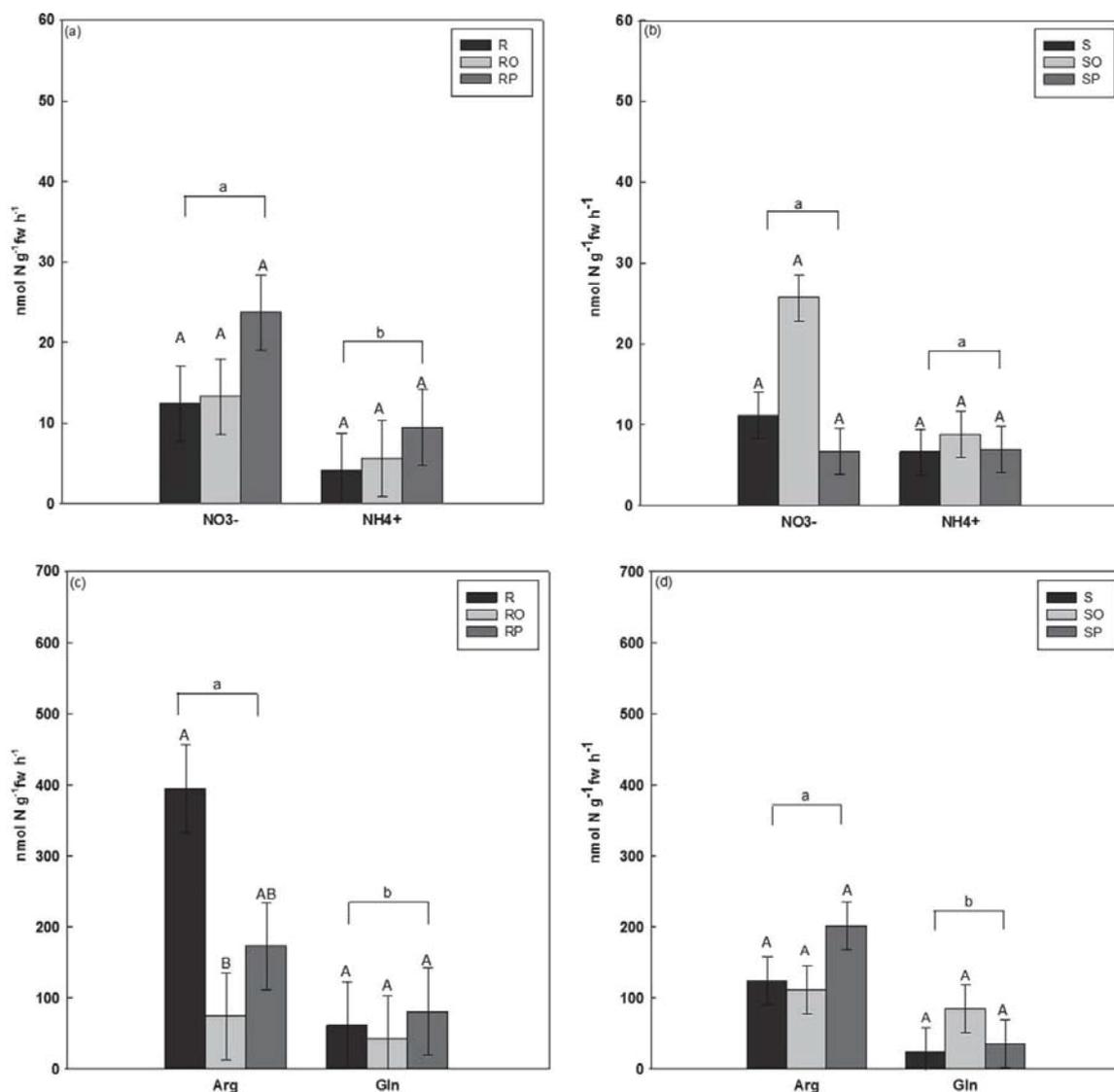


Figure 5. Net nitrogen uptake capacity [$\text{nmol N g}^{-1} \text{fw h}^{-1}$] in grapevine roots of the four different nitrogen forms (a) & (b) [NO_3^- , NH_4^+] and (c) & (d) [Arg, Gln]. Measured in the six different cultivation systems (a) & (c) R (■); RO (▒); RP (■) and (b) & (d) S (■); SO (▒); SP (■). Bars represent means \pm SE (single cropping $n = 8$, mixed cropping $n = 20$). Riesling cropping systems and Sauvignon Blanc cropping systems were analysed separately. Lower case letters indicate significant differences within a nitrogen form; capital letters indicate significant differences within a cultivation system between different N-forms, MIXED MODELS, $p \leq 0.05$. Exposition period: 2 h.

influenced by tartaric and lactic acid, while differences for Sauvignon Blanc wines were mostly caused by malic acid, total acid and sugars. The sensory analyses of the different wines indicated no significant changes in the aroma attributes when grapevine was grown in combination with trees, neither for Riesling (Figure 7(a)), nor for Sauvignon Blanc (Figure 7(b)). Only slight tendencies for differences in a few aroma attributes (e.g. mint and odour) were detected.

Discussion

Cultivation of grapevine in an AF can improve water relations at drought

The measured water status of the grapevine cultivar Riesling was affected by the AF. Cultivation with oak and poplar increased leaf water potential (Ψ_{leaf}) in Riesling but not in Sauvignon Blanc (Figures 2(a) and 2(b)), which seems to be a benefit for Riesling when grown in an AF. According to Deloire et al. (2004), there is a good relationship between the water status of plants, measured in

Table 1. Mean values of chemical attributes of the six experimental wines made from grapes of the different cultivation systems. Mixed cropping systems of the Riesling and Sauvignon Blanc varieties were separated analysed. Riesling and Sauvignon Blanc act as control. Significant differences (Riesling $n = 2$, Sauvignon Blanc $n = 1$) are marked with an asterisk (MIXED MODELS, $p \leq 0.05$). (R, Riesling; RO, Riesling/oak; RP, Riesling/poplar; S, Sauvignon Blanc; SO, Sauvignon Blanc/oak; SP, Sauvignon Blanc/poplar). ND = not detectable.

| Sample | pH | Total acid [g L ⁻¹] | Tartaric acid [g L ⁻¹] | Malic acid [g L ⁻¹] | Lactic acid [g L ⁻¹] | Sugar [g L ⁻¹] | Phenols [g L ⁻¹] |
|--------|-------|------------------------------------|---------------------------------------|------------------------------------|-------------------------------------|-------------------------------|---------------------------------|
| R | 2.51 | 11.31 | 5.03 | 3.33 | 2.73 | 3.74 | 0.148 |
| RO | 2.37 | 11.91* | 5.43 | 3.25 | 2.52 | 3.19 | 0.125 |
| RP | 2.53 | 11.15 | 5.10 | 3.09 | 2.32* | 2.75* | 0.099 |
| S | 2.66 | 13.19 | 5.48 | 4.22 | 1.76 | 3.43 | ND |
| SO | 1.74* | 12.86 | 4.91 | 4.51 | 1.70 | 4.10* | ND |
| SP | 2.52 | 12.47* | 5.19 | 3.94 | 1.90 | 3.55 | ND |

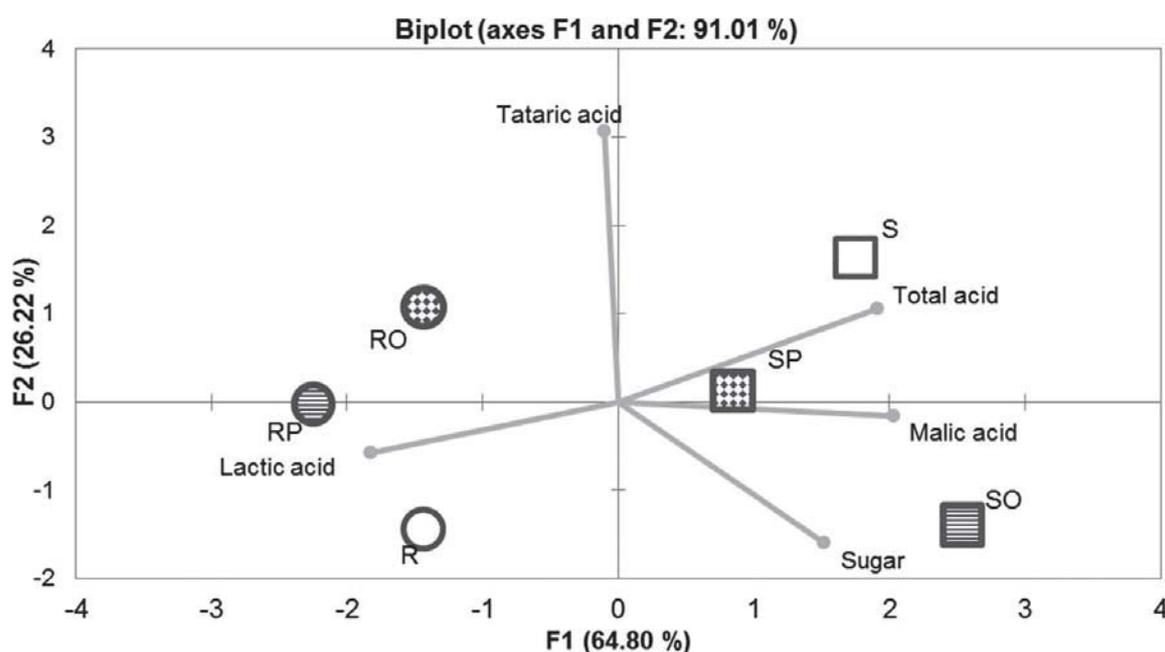


Figure 6. Principal component analysis Biplot (PCA; F1 vs F2) of the chemical attributes of the six experimental wines from grapes of the different cultivation systems (R (○), Riesling; RO (◐), Riesling/oak; RP (◑), Riesling/poplar; S (□), Sauvignon Blanc; SO (◒), Sauvignon Blanc/oak; SP (◓), Sauvignon Blanc/poplar).

terms of the leaf water potential (Ψ_{leaf}) and the available water reserves in the soil area occupied by the roots. A reduction in leaf water potential (Ψ_{leaf}) reflects lower availability of water in the soil or can even be an indication of water stress (Schultz 2003; Deloire et al. 2004). However, severe water stress did not occur during the data collection period, (see supplemental data, S.2). Grapevines close their stomata to reduce water loss; however, a prolonged closure leads to a reduced photosynthesis, reduced sugar accumulation and finally resulting in a reduced wine quality (dos Santos et al. 2007).

There was no significant difference in leaf water potential (Ψ_{leaf}) between the tree species used in this study. There was no competition for water in the AF systems, but rather the opposite, since the mixed cropping combination RO lead to a significant reduction in leaf water potential and, therefore, to an easing of competition for water. Apparently, water relations of the grapevine cultivar Riesling can be improved by AF under these conditions. According to Bayala and Wallace (1996), trees may affect the availability of water to crops in an AF by improving soil physiological properties, reducing runoff and soil surface evaporation as well as intercepting rain. Trees have a deep root system with a consistent framework of large perennial roots and many short-lived branch roots (Pallardy 2008). By contrast, roots of grapevines are mostly located in the top

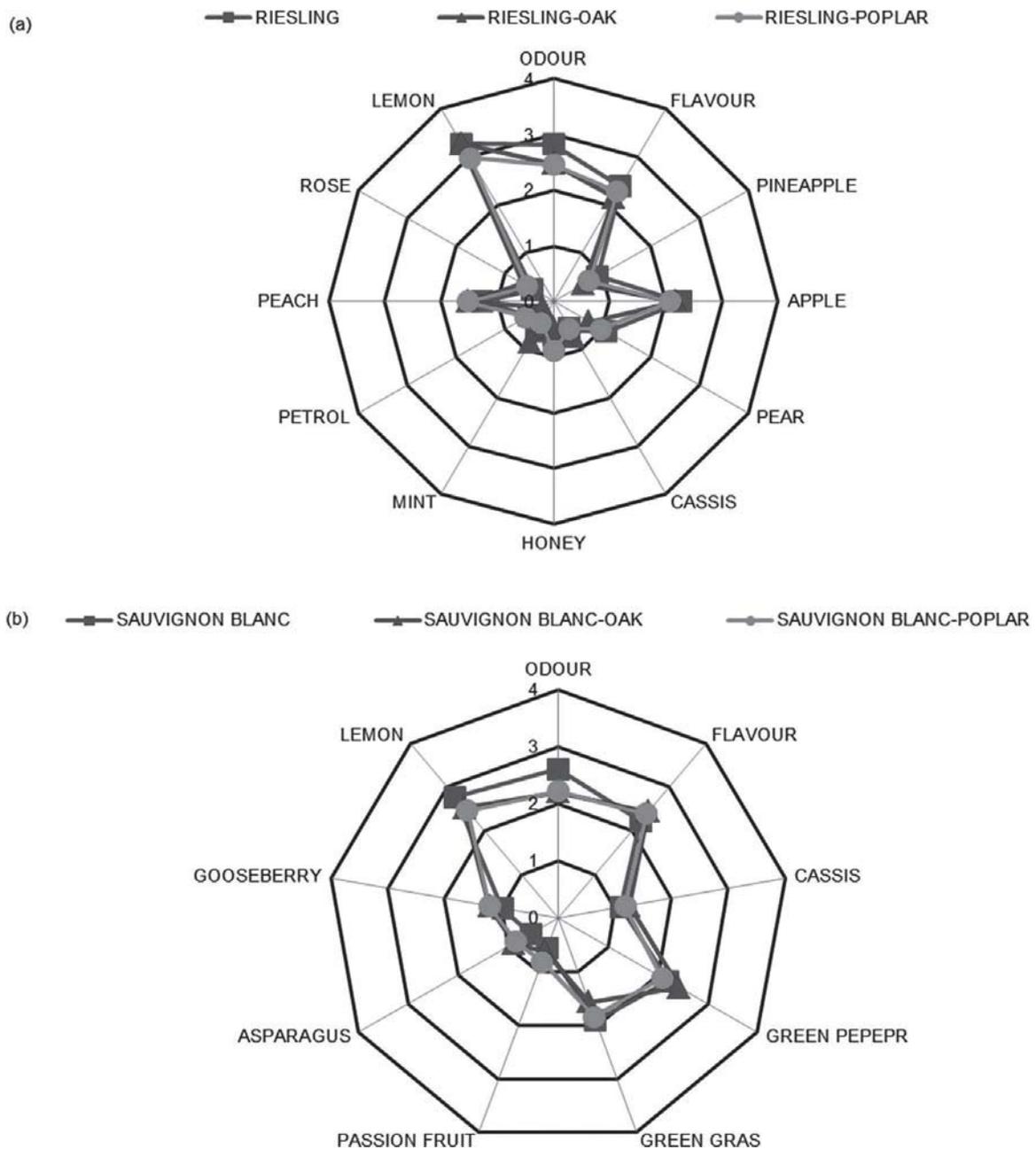


Figure 7. Aroma and sensory descriptions for the matured wines, of the different cultivation systems (a) (■) Riesling, (▲) Riesling/oak; (●) Riesling/poplar and (b) (■) Sauvignon Blanc; (▲) Sauvignon Blanc/oak; (●) Sauvignon Blanc/poplar, as determined by the tasting panel. Mean values are shown for the two appointments ($n = 10; 12$). Riesling and Sauvignon Blanc act as control.

60 cm of the soil, these fine roots do most of the water and N acquisition (Jackson 2008). Cannell et al. (1996) suggested a 'biophysical hypothesis' for agroforestry research. They assumed that beneficial effects by growing trees in combination with crops only occur when the trees were able to acquire resources like water, light and nutrients that the crops would otherwise not acquire. Overall, the present results indicated that, tree roots could provide Riesling grapevine roots with water from deeper soil layers. These phenomena is a so-called 'hydraulic lift', a process, of passive soil – water movement from deep – moist to shallow – drier soil layers, driven by the water potential gradient (Caldwell et al. 1998). In addition, trees can act as windbreakers and shielding the soil from radiation and wind (Bayala and Wallace 1996). Therefore, trees slow the movement of wind and air circulation, leading to reduced evaporative, while, the distribution and utilization of water is improved (Davis and Norman 1988; Jose et al. 2004).

The $\delta^{13}\text{C}$ method is an integrating measure for characterizing the water supply from the time of development to the time of harvest of the plant material studied (Gaudillere 2002). It is determined by the gradient of CO_2 in the atmosphere and the intercellular CO_2 concentration of the leaves (C_i/C_a). This ratio is mainly influenced by water availability (Farquhar et al. 1989). Our results did not show significant differences in the abundance of $\delta^{13}\text{C}$ in the grapevine leaves between the cultivation types (Figures 4(a) and 4(b)). This finding supports our conclusion from Figure 2, that there was no competition for water in the studied AF system. Moreover, these results clarify that, the overall water availability during the vegetation period was sufficient for the grapevines.

Cultivation in AF can improve N nutrition of grapevines

When Riesling and Sauvignon Blanc grow with trees, the total leaf N concentration significantly increased in combination with oak and slightly increased in combination with poplar (Figure 3(a) and 3(b)). The nitrogen isotope composition [$\delta^{15}\text{N}\text{‰}$] of the leaves demonstrated (Figure 4(a) and 4(b)) that the monoculture of both Riesling and Sauvignon Blanc had the highest abundances. This is an indicator for the amount of the isotope, which was taken up by the plant (Robinson 2001). At the first glance, this is contradictory to the net uptake capacities of N that were reduced in the AF systems, whereas the N concentrations of the leaves were enhanced. So far, we just can hypothesize about this discrepancy. The differences in nNUC may not reflect the actual uptake of organic and inorganic N compounds due to different availabilities in the soil. The different N contents could result from different uptake capacities. Furthermore, environmental factors have major impacts on the uptake of N by the roots. Our measurements took place at one time point in summer, but the leaves developed earlier in the vegetation period. Therefore, we cannot exclude that net uptake capacities were different between monocultures and AF system at other time points and different N-pools, with different N forms and quantities were built throughout the year. Finally, the N content of leaves in a perennial woody plant greatly depends on stored resources in the plant that are mobilized in spring (Dickson 1989; Millard and Grelet 2010). Therefore, the N content of leaves often reflects the net uptake capacities of the previous year. The net N uptake capacity of the current year rather determines the extent at which storage pools in the stem are refilled.

What can be concluded from the differences in leaf $\delta^{15}\text{N}$ abundance between monocultures and agroforestry system cultivation of grapevines?

The N isotope composition [$\delta^{15}\text{N}\text{‰}$] of the leaves is an indicator for the origin of N acquired by the plant (Robinson et al. 2000). From the results it may be concluded that the N taken up from the soil was less subjected to biological N_2 fixation by free living soil-microbes, when the grapevine was grown in combination with trees. However, the differences observed may also result from (a) different N isotope fractionation processes during uptake, transport and metabolite transformation of soil N sources (Schmidt et al. 2015) and/or (b) differences in the availability and use of inorganic versus organic N compounds in the soil (Näsholm et al. 2009). In addition, plants can change their preference for different N forms (e.g. NO_3^- versus NH_4^+) with different nitrogen isotope composition in response to environmental conditions, but also to management practices, such as fertilization or harvest, that can shift to proportionate uptake rates of NO_3^- and NH_4^+ (Högberg 1997; Pardo et al. 2002). However, the origin of the N isotopes and the $\delta^{15}\text{N}$ of the major N compounds in the xylem sap, which were taken up by the plant for N assimilation, remain unknown.

Water and nitrogen consumption of grapevine is not influenced by agroforestry cultivation

Water and fertilizer management, especially for N, are strongly linked to each other, in a way, that changes in one parameter will affect the efficiency of the other. This means, the more water is available for plants, the more available nitrogen can be taken up by the roots and therefore be

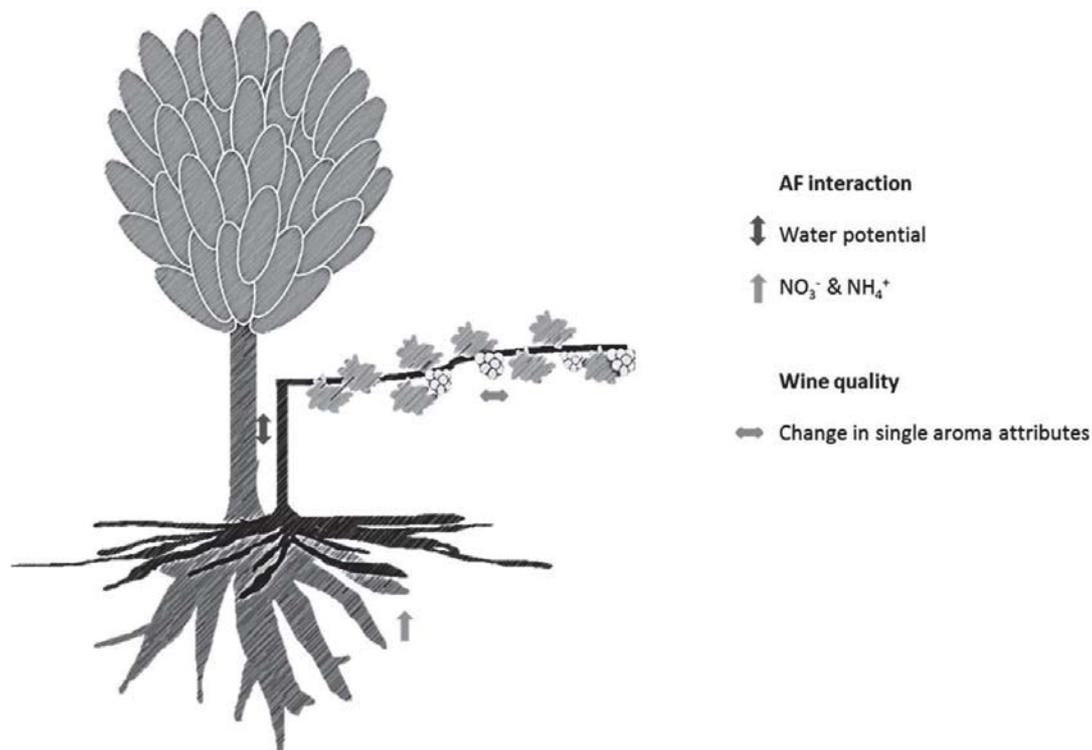


Figure 8. Schematized conclusion of the effects of trees on the grapevine in an agri-silvicultural system. Nitrate and ammonium is increased in mixed cropping system; no difference in water potential, therefore no water stress in mixed cropping system; comparable wine quality in both cropping systems. Abbreviations; blue arrow, increase in absorption level; red arrow, change in both directions; green arrow, no change.

retrievable for the plant. From a comparison of Figure 2(a), 3(a) and 3(b), this conclusion can be supported. However the extension is variety dependent. In the mixed cropping varieties of Riesling in combination with oak and poplar, the leaf water potential (Ψ_{leaf}) was significantly lower (Figure 2(a) and 2(b)) simultaneously the total leaf N-concentration was higher; similar tendencies can be seen in Sauvignon Blanc but the results are not statistically significant.

The C:N ratio in Figures 3(e) and 3(f) describe the proportion of carbon (C) and N in leaves of the six different AF systems studied. The smaller the ratio, the more N is available. This implies that the combination with the smallest C:N ratio, had the highest net N uptake, a change in water or nitrogen supply results in a C: N ratio imbalance (Chen et al. 2015). According to our results no statistical differences were detected between monoculture and mixed cropping systems, again disproving a competition for nitrogen and water. The concluding physiological influences and changes of trees on and grapevines in relation to water and nitrogen, as summarized in Figures 2–5, are shown as a schematic Figure 8.

Chemical attributes of Riesling and Sauvignon Blanc are slightly changed when grown in an AF system

The influence of the trees on the wine is variety dependent (Table 1). The PCA in Figure 6 indicates that these changes were minor (RO: total acid, RP: lactic acid and sugar; SO: pH and sugar, SP: total acid) when AF systems were compared to monocultures. Trees influenced mainly sugar and acid concentration of the wine. Nevertheless, the results indicate that the sugar to acids balance, that primary contributes to flavour (Liu et al. 2006; Conde et al. 2007), is influenced by both tree varieties. Tartaric and malic acid account for two-third or even more of all organic acids in grapes determining the pH of wine (Kliewer 1966; Waterhouse et al. 2016). To conclude, after this first hint, more studies have to be done to exactly clarify which tree may influence which wine parameter.

The aroma and sensory evaluation of Riesling (Figure 7(a)) and Sauvignon Blanc (Figure 7(b)), indicate only small tendencies towards an increase or decrease of single aroma compounds. Habran et al. (2016) reported that a mild water deficit and moderate N availability affected berry metabolism towards the synthesis of phenolic and aroma compounds. Chapman et al. (2005) and Habran et al. (2016) found that vegetal aroma contributes, especially bell-pepper, is reduced under water deficit whereas fruity aroma descriptors were higher under these conditions. However, this cannot be proven because there was no water stress for the vines, and there were no significant differences detected in tasting. Only tendencies prove small changes in the aroma components of the wine (Figure 7). Several hundreds of volatile compounds contribute to wine aroma, with concentrations ranging from several mg L⁻¹ to a few ng L⁻¹, sometimes even less (Francis and Newton 2005; Conde et al. 2007). The olfactory threshold and the perception of these compounds can vary considerably. Many complex mechanisms that are involved in the development of aroma; these may include biochemical, cultural and enzymatic factors, as well as viticultural management practices during growth, processing and fermentation (González-Barreiro et al. 2015). This is the reason, why aromas are difficult to study (Francis and Newton 2005; Ribéreau-Gayon et al. 2006). As a quintessence only slightly differences of the chemical attributes and the aroma components of the wines were detected in the AF systems (Table 1; Figures 6 and 7).

Conclusions

Mixed cropping systems of grapevines (*Vitis vinifera* L. cv. Riesling and *Vitis vinifera* L. cv. Sauvignon Blanc) with oak (*Quercus petraea*) and poplar (*Populus tremula* × *P. alba*) revealed that the presence of the trees increased leaf water potential Ψ_{leaf} of the neighbored Riesling but not of Sauvignon Blanc. Furthermore, N availability and acquisition by grapevines increased with cultivation in combination with trees in agroforestry systems. We conclude from these outcomes that, at least under the conditions of this study, there was no competition in this type of agri-silvicultural system with regard to water and N in two different years. Rather, the different plant species supported each other in their net N uptake capacity. In addition, trees do not significantly affect quality-associated chemical attributes of the wines and their related quality. The sensory attributes of the wines were similarly good in both cultivation systems. These findings suggest that an agri-silvicultural system could be useful for practical implementation towards a resource-preserving production of high quality wine.

Acknowledgments

We thank Elisabeth Voigt and Stefan Schäfer for sample collection and Cornelia Blessing, as well as Dr. Baoguo Du from the University of Freiburg for technical assistance. Further thanks to Mr. Büdinger as well as to the wineries Lauer, Eilenz, Weber and Vols for maintenance of the vineyard system and the on-site support in Ayl. The sensory panel of the University of Hohenheim was recently thanked.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Alleweldt G, Eibach R, Rühl E. 1982. Untersuchungen zum Gaswechsel der Rebe I. Einfluß von Temperatur, Blattalter und Tageszeit auf Nettphotosynthese und Transpiration [Studies on the gas exchange of the vine I. Influences of temperature, leaf age and time of day on net photosynthesis and transpiration.]. *Vitis*. 21:93–100.
- Altieri MA, Nicholls CI. 2002. The simplification of traditional vineyard based agroforests in northwestern Portugal: some ecological implications. *Agroforestry Systems*. 56:185–191.

- Bainard LD, Klironomos JN, Gordon AM. 2011. Arbuscular mycorrhizal fungi in tree-based intercropping systems: A review of their abundance and diversity. *Pedobiologia*. 54:57–61.
- Bayala J, Wallace JS. 1996. The water balance of mixed tree-crop systems. In: Ong CK, Huxley P, eds. *Tree-crop interactions: A physical approach*. Wallingford (UK): CAB International; p. 189–233.
- Bell SJ, Henschke PA. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust J Grape Wine Res*. 11:242–295.
- Caldwell MM, Dawson TE, Richards JH. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia*. 113:151–161.
- Cannell MGR, Van Noordwijk M, Ong CK. 1996. The central agroforestry hypothesis: the trees must acquire resources that the crop would not otherwise acquire. *Agrofor Syst*. 34:27–31.
- Chapman DM, Roby G, Ebeler SE, Guinard JX, Matthews MA. 2005. Sensory attributes of Cabernet Sauvignon wines made from vines with different water status. *Aust J Grape Wine Res*. 11:339–347.
- Chaves MM, Zarrouk O, Francisco R, Costa JM, Santos T, Regalado AP, Rodrigues ML, Lopes CM. 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann Bot*. 205:661–676.
- Chen D, Wang S, Xiong B, Cao B, Deng X. 2015. Carbon/nitrogen imbalance associated with drought-induced leaf senescence in *sorghum bicolor*. *PLoS One*. 10(8):e0137026.
- Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food*. 1:1–22.
- Coplen TB, Brand WA, Gehre M, Gröning M, Meijer HAJ, Toman B, Verkouteren RM. 2006. New Guidelines for $\delta^{13}\text{C}$ Measurements. *Anal Chem*. 78:2439–2441.
- Davis JE, Norman JM. 1988. Effects of shelter on plant water use. *Agric Ecosyst Environ*. 22/23:393–405.
- Deloire A, Carbonneau A, Wang Z, Ojeda H. 2004. Vine and water: a short review. *J Int Sci Vigne Vin*. 38:1–13.
- Deluc LG, Quilici DR, Decendit A, Grimplet J, Wheatley MD, Schlauch KA, Jm M, Cushman JC, Cramer GR. 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of cabernet sauvignon and chardonnay. *BMC Genomics*. 10. doi:10.1186/1471-2164-10-212
- Dickson RE. 1989. Carbon and nitrogen allocation in trees. *Ann Sci For*. 46:631–647.
- Dos Santosdos TP, Cm L, MI R, De Souza CR, Ricardo-da-Silva JM, Maroco JP, Pereira JS, Chaves M. 2007. Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines. *Sci Hortic*. 112:321–330.
- Eichhorn MP, Paris P, Herzog F, Incoll LD, Liagre F, Mantzanas K, Mayus M, Moreno G, Papanastasis VP, Pilbeam DJ, et al. 2006. Silvoarable systems in Europe – past, present and future prospects. *Agrofor Syst*. 67:29–50.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol*. 40:503–537.
- Francis IL, Newton JL. 2005. Determining wine aroma from compositional data. *Aust J Grape Wine Res*. 11:114–126.
- Gaudillere JP. 2002. Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. *J Exp Bot*. 53:757–763.
- Gessler A, Kreuzwieser J, Dopatka T, Rennenberg H. 2002. Diurnal courses of ammonium net uptake by the roots of adult beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees. *Plant Soil*. 240:23–32.
- Gessler A, Schneider S, Von Sengbusch S, Weber P, Huber S, Rothe A, Rennenberg H. 1998. Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New Phytol*. 138:275–285.
- González-Barreiro C, Rial-Otero R, Cancho-Grande B, Simal-Gándara J. 2015. Wine aroma compounds in grapes: a critical review. *Crit Rev Food Sci Nut*. 55:202–218.
- Habran A, Commisso M, Helwi P, Hilbert G, Negri S, Ollat N, Gomès E, Van Leeuwen C, Guzzo F, Delrot S. 2016. Rootstocks/scion/nitrogen interactions affect secondary metabolism in the grape berry. *Front Plant Sci*. doi:10.3389/fpls.2016.01134
- Hanson EJ, Howell GS. 1995. Nitrogen accumulation and fertilizer use efficiency by grapevines in short-season growing areas. *HortScience*. 30:504–507.
- Högberg P. 1997. Transley review no. 95 ^{15}N natural abundance in soil-plant systems. *New Phytol*. 137:179–201.
- Hu B, Simon J, Kuster TM, Arend M, Siegwolf R, Rennenberg H. 2013. Nitrogen partitioning in oak leaves depends on species, provenance, climate conditions and soil type. *Plant Biol*. 15:198–209.
- Jackson RS. 2008. *Wine science. Principles and applications*. Third ed. Amsterdam (Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Sydney, Tokio): Elsevier Inc.
- Jose S. 2009. Agroforestry for ecosystem services and environmental benefits: an overview. *Agrofor Syst*. 76:1–10.
- Jose S, Gillespie AR, Pallardy SG. 2004. Interspecific interactions in temperate agroforestry. *Agrofor Syst*. 61:237–255.
- Keller K. 2005. Deficit irrigation and vine mineral nutrition. *Am J Enol Vitic*. 53:267–283.
- Kliwer WM. 1966. Sugars and organic acids of vitis vinifera. *Plant Physiol*. 41:923–931.
- Kreuzwieser J, Föhn S, Rennenberg H. 2002. Impact of waterlogging on the N-metabolism of flood tolerant and non-tolerant tree species. *Plant Cell Environ*. 25:1039–1049.
- Liu HF, Wu BH, Fan PG, Li SH, Li LS. 2006. Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *J Sci Food Agric*. 86:1526–1536.

- Lovisol C, Lavoie-Lamoureux A, Tramontini S, Ferrandino A. 2016. Grapevine adaptations to water stress: new perspectives about soil/plant interactions. *Theor Exp Plant Physiol.* 28:53–66.
- Lovisol C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A. 2010. Drought-induced changes in development and function of grapevine (*Vitis spp.*) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Funct Plant Biol.* 37:98–116.
- Lundgren BO, Raintree JB. 1983. Sustained agroforestry. *Agricultural Research for Development: Potentials and Challenges in Asia.* ISNAR, The Hague, ICRAF Reprint 3:1–26.
- Mast MN, Hernandez R, French WT, Claupein W, Graeff-Hönninger S. 2015. Characterization of different biomasses based on their sugar profile with focus on their utilization for microbial biodiesel production. *Int J Green Energy.* 2015(12):930–938.
- Mendes-Ferreira A, Barbosa C, Lage P, Mendes-Faia A. 2011. The impact of nitrogen on yeast fermentation and wine quality. *CIENC TEC VITIVINIC.* 26:17–32.
- Millard P, Grelet GA. 2010. Nitrogen storage and remobilization by trees. *Tree Physiol.* 30:1083–1095.
- Näsholm T, Kielland K, Ganetey U. 2009. Uptake of organic nitrogen by plants. *New Phytol.* 182:31–48.
- Nerlich K, Graeff-Hönninger S, Claupein W. 2013. Agroforestry in Europe: a review of the disappearance of traditional systems and development of modern agroforestry practices with emphasis an experience in Germany. *Agrofor Syst.* 87:475–492.
- Pallardy SP. 2008. *Physiology of Woody Plants.* Third ed. Amsterdam (Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Sydney): Tokio. Elsevier Inc.
- Pardo LH, Hemon HF, Monzoya JP, Fahey TJ, Siccama FG. 2002. Response of the natural abundance of ^{15}N in forest soil and foliage to high nitrate loss following clear-cutting. *Can J For Res.* 32:1126–1136.
- Qi H, Coplen TB, Geilmann H, Brand WA, Böhlke JK. 2003. Two new organic reference materials for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements and a new value for the $\delta^{13}\text{C}$ of NBS 22 oil. *Rapid Commun Mass Spectrom.* 17:2483–2487.
- Rennenberg H, Dannenmann M. 2015. Nitrogen nutrition of trees in temperate forests—the significance of nitrogen availability in the pedosphere and atmosphere. *Forests.* 6:2820–2835.
- Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdiou D. 2006. *Handbook of enology: the chemistry of wine stabilization and treatments, volume 2.* 2nd ed. West Sussex (England): John Wiley & Sons Ltd.
- Ringnér M. 2008. What is principal component analysis? *Nat Biotechnol.* 26:303–304.
- Robinson D. 2001. $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle. *Trends Ecol Evol.* 16:153–162.
- Robinson D, Handley LL, Scrimgeour CM, Gordon DC, Forster BP, Ellis RP. 2000. Using stable isotope natural abundances ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. *J Exp Bot.* 51:41–50.
- Schmidt HL, Robins RJ, Werner RA. 2015. REVIEW ARTICLE Multi-factorial in vivo stable isotope fractionation: causes, correlations, consequences and applications. *Isotopes Environ Health Stud.* 51:155–195.
- Schmitt A. 1983. *Aktuelle weinanalytik. ein leitfaden für die Praxis.* [Current wine analysis. A practical guideline]. 2nd ed. Germany: Schwäbisch Hall. Heller Chemie- und Verwaltungsgesellschaft mbH.
- Scholander PF, Bradstree ED, Hemmingsen EA, Hammel HT. 1965. Sap pressure in vascular plants. *Science.* 148:339–346.
- Schultz HR. 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. Cultivars during Drought. *Plant Cell Environ.* 26:1393–1405.
- Simon J, Dannenmann M, Gasche R, Holst J, Mayer H, Papen H, Rennenberg H. 2011. Competition for nitrogen between adult European beech and its offspring is reduced by avoidance strategy. *For Ecol Manage.* 262:105–114.
- Singleton VL, Orthofer R, Lamuela-Raventós RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* 299:152–178.
- Van Leeuwen C, Tregoat O, Choné X, Bois B, Pernet D, Gaudillère JP. 2009. Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *J Int Sci Vigne Vin.* 43:121–134.
- Vin Anchor. 2000. *S. cerevisiae;* Anchor, Johannesburg, South Africa.
- Waterhouse AL, Sacks GL, Jeffery DW. 2016. *Understanding wine chemistry.* The Atrium (Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom): John Wiley & Sons, Ltd, John Wiley & Sons, Ltd.