



Review

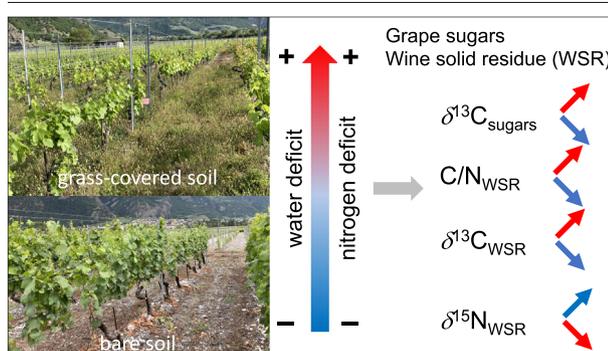
Soil management affects carbon and nitrogen concentrations and stable isotope ratios in vine products

Jorge E. Spangenberg^{a,*}, Vivian Zufferey^b^a Institute of Earth Surface Dynamics (IDYST), University of Lausanne, CH-1015 Lausanne, Switzerland^b Research Center of Viticulture, Agroscope, CH-1009 Pully, Switzerland

HIGHLIGHTS

- Vines were grown on bare and grass-covered soils with and without irrigation.
- $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C/N were determined in freeze-dried wines from all treatments.
- Cover crops increase vine water deficiency and ^{13}C enrichment in vine products.
- Cover crops compete for N and force vines to use ^{15}N -depleted organic reserves.
- Nitrogen and water deficits in cover-cropped vines are alleviated by irrigation.

GRAPHICAL ABSTRACT



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ABSTRACT

Weeds reduce vineyard productivity and affect grape quality by competing with grapevines (*Vitis vinifera* L.) for water and nutrients. The increased banning of herbicides has prompted the evaluation of alternative soil management strategies. Cover cropping seems to be the best alternative for weed management. However, it may impact vine growth, grape yield, and quality. Quantitative studies on these changes are scarce. Our study aimed to investigate the combined effect of grass cover and water availability on vines of three cultivars, the white Chasselas and Petite Arvine and the red Pinot noir field-grown under identical climatic and pedological conditions and grafted onto the same rootstock. Soil management and irrigation experiments were performed during the 2020–2021 seasons. Two extreme soil management practices were established in the vineyard, based on 100 % bare soil (BS) by the application of herbicides with glufosinate or glyphosate as active ingredients and 100 % grass-covered soil (GS) by cover cropping with a mixture of plant species. Two water statuses were imposed by drip irrigation (DI) and no irrigation (NI). The level of vine-weed competition for water and nitrogen (N) was assessed in the vine, must, and wine solid residues (WSRs) by comparing measurements, i.e., the yeast assimilable N content, $\text{C}/\text{N}_{\text{WSR}}$, carbon and N isotope ratios ($\delta^{13}\text{C}_{\text{grape-sugars}}$, $\delta^{13}\text{C}_{\text{WSR}}$, and $\delta^{15}\text{N}_{\text{WSR}}$) among the different treatments (BS-DI, BS-NI, GS-DI, GS-NI). The increase in the $\delta^{13}\text{C}_{\text{grape-sugars}}$ and $\delta^{13}\text{C}_{\text{WSR}}$ values with increasing plant water deficit mimicked the observations in irrigation experiments on BS. The N_{WSR} content and $\delta^{15}\text{N}_{\text{WSR}}$ values decreased with water stress and much more strongly in vines on GS. The dramatic N deficit in rainfed vines on GS could be alleviated with irrigation. The present study provides insights from chemical and stable isotope analyses into the potential impact of cover cropping in vineyards in the context of the banning of herbicides in a time of global water scarcity due to climate change.

* Corresponding author.

E-mail address: Jorge.Spangenberg@unil.ch (J.E. Spangenberg).

Contents

1. Introduction	2
2. Materials and methods	3
2.1. Experimental site, vine cultivars, and experimental design	3
2.2. Measurement of the grapevine water and N status	4
2.3. Wine making, wine solid residues and C and N isotope analysis	4
3. Results and discussion	5
3.1. Vine water status	5
3.2. Vine nitrogen status	6
3.3. Carbon isotope composition of wine solid residues	8
3.4. C/N molar ratios and N isotope ratios of wine solid residues	9
4. Conclusions	13
CRediT authorship contribution statement	13
Data availability	14
Declaration of competing interest	14
Acknowledgments	14
References	14

1. Introduction

Grapevine (*Vitis vinifera* L.) is a major crop of high economic value worldwide. In the context of climate warming, grapevines, similar to most crops, experience increasingly frequent, more intense, and longer water deficits during periods of extreme heat. Concurrently, viruses, bacteria, fungi, and insects have deleterious effects on berry development, resulting in low yields and poor-quality fruits (Reineke and Thiery, 2016; Pertot et al., 2017). Therefore, in conventional viticulture, pesticides, fungicides, and herbicides are used to ensure high-quality products. Weeds, grass, and other plants in vineyards may compete with the vines for water and nutrients, affecting vine growth and productivity (Ingels et al., 2005; Muscas et al., 2017). The most widely used nonselective and broad-spectrum herbicides in vineyards contain the active ingredient glufosinate (2-amino-4-[hydroxy (methyl-phosphonyl)]butanoic acid) or glyphosate (*N*-phosphonomethyl glycine) (Zaller et al., 2018; Mandl et al., 2018; Takano and Dayan, 2020). The intensive use of glyphosate was reported to accelerate the development of resistant weed species (Sammons and Gaines, 2014; Heap and Duke, 2018). Glufosinate is used in vineyards mainly to manage glyphosate-resistant weeds. The proven acute toxicity of glufosinate to humans and the environment (Zaller et al., 2018; Takano and Dayan, 2020) led to the banning of this herbicide in the European Union and Switzerland in 2020. The ecotoxicological threats posed by glyphosate to the environment and living organisms have been described in previous studies (Mann and Bidwell, 1999; Busse et al., 2001; Gill et al., 2018; Tarazona et al., 2017). The World Health Organization classified glyphosate as being probably carcinogenic to humans in 2015 (van Bruggen et al., 2018). To our knowledge, no substitute for glufosinate or glyphosate with low (or no) toxicity to the environment and living organisms is available for weed management. Therefore, the European Union and Switzerland reapproved the use of glyphosate for another five years in 2017 (van Straalen and Legler, 2018). Farmers must now look for agroecological alternatives to toxic herbicides.

Conventional weed control in vineyards relies on herbicides and tillage. Ecological alternatives to herbicides include mechanical weeding, shallow tillage (mechanical agitation of the soil), grass cutting, grazing by farm animals, soil mulching, and cover cropping. All of these are traditional techniques that have been used to manage weeds and soil surfaces before the development of herbicides. Mechanical weed control and soil management using tractors with various implements for weeding, below-surface tilling, above-surface tilling, and grass-cutting mechanisms may require high capital cost. Weeding machines can damage vine trunks and roots and are particularly challenging in sloping and mountain vineyards (Keller, 2015). Additionally, tilling disturbs the soil structure, physical properties, and microorganisms and triggers soil organic matter degradation and erosion (Raclot et al., 2009; Blavet et al., 2009; Capello et al., 2019). Periodic soil tillage alone may lead to a high erosion rate and nutrient loss in vineyards

on steep slopes or mountains (Biddoccu et al., 2016; Mirás-Avalos et al., 2020). Additionally, frequent machinery traffic on sloping vineyards affects the spatial distribution of soil physical properties and water availability due to topsoil and subsoil compaction (Ferrero et al., 2005). Farm grazing animals (sheep, cattle) may be integrated into vineyards during vine dormancy to reduce the use of herbicides (by 60 %), machines, fuel, and labor and enhance soil organic matter and fertility (Niles et al., 2017; Goncalves et al., 2021; Schoof et al., 2021). Mulching involves maintaining a permanent or semipermanent protective and permeable cover on the soil surface. Mulches can be composed of different materials, such as gravel, wood chips, straw (e.g., barley straw), crop residues, hay, crushed glass, distillers grains, or plastic mulching films made from polypropylene or polyester (Nachtergaele et al., 1998; Bond and Grundy, 2001; Hostetler et al., 2007; Prosdocimi et al., 2016; Lopez-Urrea et al., 2020; Olejar et al., 2021). This cover keeps the ground surface clear, blocks light from reaching germinating weeds, limits weed growth, and reduces soil erosion and nutrient leaching (Prosdocimi et al., 2016 and references therein). Mulches can be used to weed management in vineyards and may positively affect soil temperature and hydraulic characteristics (water infiltration and evaporation), as well as vine growth and yield (Buesa et al., 2020). Soil plastic mulching may have a significant water-saving effect (Nouri et al., 2019). These eventual advantages depend on the mulch material, soil characteristics (depth, water content, organic matter content, and fertility), and climate (Nachtergaele et al., 1998; Bavougian and Read, 2018). The potential disadvantages of mulching are the challenges in obtaining mulch material, transportation costs, initial installation costs, and labor costs (Guerra and Steenwerth, 2012; Wezel et al., 2014; Bavougian and Read, 2018).

Cover cropping is an effective practice for soil management in vineyards (Tescic et al., 2007; Abad et al., 2020). It consists of replacing an unmanageable weed population with convenient, controllable cover crop species, which may yield an economic return (Teasdale, 1996). In vineyards, the soil under vines and between vine rows can be covered by crop species that remain alive for part or all of the vine growing season. Cover crop competition for water and nutrients is commonly used to mitigate vine vegetative development or vigor (Muscas et al., 2017). Competition between grapevines and cover crops for soil water and nitrogen (N), particularly in spring, when both plant types are actively growing (bloom and berry-set vine stages), can lead to severe water stress and reduce grape yield (Tescic et al., 2007). However, cover crops protect hill-slope and mountain vineyards from soil erosion and the leaching of nutrients and organic matter by runoff (Bond and Grundy, 2001). Additionally, cover crops provide many services to vineyards, including reducing soil compaction, increasing soil microbial biodiversity, and enhancing soil organic carbon (C) and nutrients (Ingels et al., 2005; Gouthu et al., 2012; Vukicevich et al., 2016; Garcia-Diaz et al., 2018; Hendgen et al., 2018).

The success of weed suppression by cover crops depends on the weed species and the choice of cover plants (Hartwig and Ammon, 2002; Delpuech and Metay, 2018). Cover crop choices include a broad range of plants that grow naturally or spontaneously or plants that are managed (i.e., mowed or soil-incorporated seeds), typically perennials, annually self-seeding species, temporary or permanent grasses, legumes, or a mix of grasses and broadleaf plants (Hartwig and Ammon, 2002). Some cover crops may mitigate water runoff and provide water to grapevines by contributing to soil water refilling (Garcia et al., 2018). The benefits of cover cropping have been shown in vineyards in regions with a temperate climate with soil fed by abundant rainfall (Trigo-Córdoba et al., 2015; Lopez-Vicente et al., 2020) and in Mediterranean semiarid regions when drip irrigation was applied (Steenwerth et al., 2013; Abad et al., 2020; Lopez-Urrea et al., 2020; Martinez et al., 2021). These last water-deficient and highly vulnerable environments have become increasingly susceptible to the impacts of climate change. Additionally, in water-deficient soils, soil nutrient uptake by plants is strongly reduced, further triggering the vine/cover-crop competition for nutrients (Celette et al., 2009). Nitrogen is one of the critical mineral nutrients for grapevines. It is used by plants to synthesize plant amino acids (AAs), N availability mediates grapevine growth, development, and metabolism. In particular, the content and composition of yeast assimilable N (YAN) affect the kinetics and completion of the alcoholic fermentation and the formation of flavor-active metabolites, which determine the color and organoleptic properties of wines (Bell and Henschke, 2005). Low N availability and uptake decrease vine vigor, grape berry size, and yield. It also increases the sugar content and the anthocyanin and tannin concentrations, which may favor the quality of the berry juice (must), specifically for red winemaking (Matthews et al., 1990; Hilbert et al., 2003).

We recently reported that the C isotope composition ($\delta^{13}\text{C}$ values) of grape sugars (glucose and fructose) at harvest is highly correlated with the predawn leaf water potential (Ψ_{pd}) measured during the veraison-harvest period for the grapevine cultivars Chasselas, Petite Arvine, and Pinot noir in irrigation experiments during six seasons (2009–2014) in Valais, Switzerland (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018). This link between vine water status and $\delta^{13}\text{C}_{\text{sugars}}$ values remained unchanged in the resulting wines and could be traced to wine volatile compounds (Spangenberg et al., 2017), wine solid residues (WSRs) (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018), and whole wine and wine ethanol (Spangenberg and Zufferey, 2019). We showed that the N isotope composition ($\delta^{15}\text{N}$ values) and content ratio of the solid residues were also well correlated with Ψ_{pd} for the same white and red wines. Building on these findings, this study aimed to assess how combined ground management and soil water status affect the C isotope composition of must sugars and the C and N isotope composition of the WSRs ($\delta^{13}\text{C}_{\text{sugars}}$, $\text{C}/\text{N}_{\text{WSR}}$, $\delta^{13}\text{C}_{\text{WSR}}$, and $\delta^{15}\text{N}_{\text{WSR}}$ values) of the same previously studied white and red grapevine cultivars (Chasselas, Petite Arvine, Pinot noir). We expect these results to show clear trends and provide quantitative insight into the effects of cover cropping and soil water deficit on wines derived from vineyards in a Swiss Alpine valley with extreme summer droughts and cold winter periods. This study provides a dual stable isotope-based comparison of the presence and absence of a vineyard soil cover crop. One of the many added benefits of using 100 % grass cover is that herbicides use is not required. The findings will certainly deepen our understanding of how the competition of cover crops for water and nutrients impacts the C and N dynamics in the soil–water–plant system of vineyards.

2. Materials and methods

2.1. Experimental site, vine cultivars, and experimental design

The trials were performed in the vineyards of the Swiss Institute of Plant Production Sciences (Agroscope) experimental station located at Leytron (46°11'N; 7°10'E, 525 m above sea level) in the Canton of Valais, Switzerland, in 2020 and 2021. The Leytron site is in an alpine valley on an alluvial cone formed by Quaternary torrential alluvial deposits

containing fragments of calcareous schists, sandstones, and marls. Leytron has a mild continental climate with hot and dry summers and is in the Cfb category according to the Köppen climate classification. The average annual precipitation and temperature of the most recent 30-year normals (1981–2010) for Valais were 603 ± 12 mm (average \pm one standard error, 1 SE) and 10.1 °C, respectively (Table 1). The temperature and precipitation values were measured at the meteorological station located at the experimental site (Leytron meteorological station, www.agrometeo.ch).

The stony-gravelly soil (peyrosol) of the vineyard is well-drained, and the water-holding capacity is approximately 150 mm in the whole soil profile (Zufferey et al., 2017). Water runoff and soil erosion are very low at the experimental site, mainly due to a nearly zero slope area and relatively low rainfall. The soil depth, structure, water-holding capacity, and fertility are uniform at the vineyard scale (soil chemical analyses in the Supplementary Table S2 of Spangenberg et al., 2020).

The experimental design included two soil management practices, two soil water statuses, and three *Vitis vinifera* L. cultivars, including the grapevines Chasselas, Petite Arvine, and Pinot noir. The white vines Chasselas (clone 14/33-4) and Petite Arvine (massale selection) and the red vine Pinot noir (clone 9/18) were grafted onto *Vitis berlandieri* \times *Vitis riparia* cv. Kober 5BB rootstock in 1995. The vines were planted in a single Guyot training system (vertical shoot position), trellised on wires, with 60 cm trunk height, and pruned to six shoots per plant. The planting density was 5500 vines ha^{-1} , and the vines were spaced 1.8×1.0 m (row spacing \times vine spacing) apart in east–west oriented rows (Zufferey et al., 2018). All the vines were 25 years old at the start of the experiment in 2020.

The vines were grown under the same natural environmental conditions, i.e., the same soil and climate components, such as light intensity and duration, air and soil temperature, air humidity, and wind speed. The vines of the white grape cultivars Chasselas and Petite Arvine were subjected annually to a foliar urea treatment, with weekly applications of 5 kg N ha^{-1} for four weeks during the onset of ripening (veraison), corresponding to days of the year (DOY) 220 and 240. The urea-based fertilizer had almost stoichiometric C and N levels (19.7 wt% C and 44.8 wt% N), a $\delta^{13}\text{C}$ value of -40.01 ± 0.03 mUr and a $\delta^{15}\text{N}$ value of -2.35 ± 0.09 mUr (Supplementary Table S2 of Spangenberg et al., 2020). The content of natural N in the soil was sufficient to support the Pinot noir plant requirements without the addition of N fertilizer (Spring et al., 2012).

Similar to previous irrigation experiments, each treatment had 40 plants in a randomized four-block design of 10 vines per block (Zufferey et al., 2018). Two extreme soil management practices were applied to the undervine and interrow soils: 100 % bare soils (BS) and 100 % grass-

Table 1

Climate data at the experimental site of Leytron (Valais, Switzerland) during 2020 and 2021^a compared to the 30-year normals (1981–2010) mean \pm standard error (SE) of the mean (data available at www.agrometeo.ch).

Month	Monthly precipitation rate (mm)			Monthly mean temperature (°C)		
	2020	2021	1981–2010 mean \pm SE	2020	2021	1981–2010 mean \pm SE
January	25	116	51 ± 6	2.3	0.3	-0.1 ± 0.4
February	81	35	47 ± 10	6.1	5.8	1.8 ± 0.3
March	82	40	42 ± 7	7.2	7.3	6.5 ± 0.3
April	25	16	35 ± 8	14	10.2	10.4 ± 0.4
May	50	100	49 ± 10	16.2	12.5	14.9 ± 0.3
June	68	44	54 ± 8	18.9	20.4	18.1 ± 0.4
July	31	139	58 ± 6	21.6	19.5	20.1 ± 0.3
August	112	49	57 ± 6	20.6	18.9	19.2 ± 0.2
September	37	43	44 ± 9	17.1	17.4	15.2 ± 0.3
October	82	20	52 ± 7	10.2	10.7	10.3 ± 0.2
November	5	16	52 ± 7	6.4	4.8	4.3 ± 0.2
December	39	108	64 ± 9	2.5	0.2	0.6 ± 0.3
Year	627	726	603 ± 12	11.9	10.7	10.1 ± 0.2

^a The 2020 growing season (April–October) was characterized by a hot and dry summer and in 2021 by a cooler spring and relatively hot and humid summer with late frosts.

covered soils (GS). The BS blocks had fully weed-free grounds obtained by chemical weeding (no tillage), with four applications of herbicide between April and July per season. In 2020, the herbicide Basta® with the active ingredient glufosinate (200 g L⁻¹) was used at a dose of 4 L ha⁻¹ per application. Glufosinate was banned in the European Union and Switzerland in 2020. Therefore, in 2021, the herbicide Roundup® with the active ingredient glyphosate (450 g L⁻¹) was used at a dose of 5 L ha⁻¹ per application. The GS treatment consisted of covering the undervine and interrow soils with a mixture of selected permanent species (no tillage). The floristic composition of the cover crop consisted of a mixture (MCS4 mixture, Delabays et al., 2016) of six selected plant species, i.e., *Bromus tectorum*, *Poa compressa*, *Medicago lupulina*, *Lotus corniculatus*, *Sanguisorba minor*, and *Brunella vulgaris*. The two soil–plant water status regimes were obtained by drip irrigation (DI) with a weekly feed of 9 L m⁻² between flowering and fruit ripening and no irrigation (NI) with natural rainfall as the only soil water supply throughout the growing season. The DI vines were well watered during the growing season, and the rain-fed NI vines were potentially water-stressed in spring–summer. Four treatments were applied to the Chasselas, Petite Arvine, and Pinot noir vines by combining the soil management practices (BS, GS) with the soil water regimes (DI, NI): BS-DI, BS-NI, GS-DI, and GS-NI. All physiological (e.g., stem water potential, chlorophyll index) and agronomic (e.g., sugar content, acidity, yeast assimilable N of the grape juice) measurements were performed in replicate vines from each block per treatment, variety, and growing season.

2.2. Measurement of the grapevine water and N status

The vine water status was assessed by measuring the stem water potential (Ψ_{stem} in megapascal, MPa) and the C isotope composition of the grape sugars ($\delta^{13}\text{C}_{\text{sugars}}$ in mUr vs. VPDB, see Section 2.3). The midday Ψ_{stem} was shown, compared to the predawn leaf water potential (Ψ_{pd}), to be the most discriminating and sensitive indicator for moderate and severe plant water deficits (Chone et al., 2001). The midday Ψ_{stem} measurements were performed using a pressure chamber (Model 3005 Soil Moisture Equipment Corp., Santa Barbara, CA, USA) between 1400 and 1500 GMT, when evapotranspiration was at a maximum. The measurements were performed weekly, from bloom (June) to harvest (September), on two single mature, undamaged, nonsenescent, and nontranspiring leaves from two vines of each block per treatment. The Ψ_{stem} values reported here are average values of all technical replicates measured during the veraison–harvest period in the four biological replicates per treatment. For non-water-stressed grapevines, the Ψ_{stem} value is higher than -0.6 MPa; for values between -0.6 and -0.9 MPa to -0.9 and -1.1 MPa, the water deficit is low to moderate, respectively, and when the Ψ_{stem} values are lower than -1.1 MPa, the plant is highly water-stressed (van Leeuwen et al., 2009). Additionally, the Ψ_{pd} was measured two times during veraison–harvest (results in Supplementary Tables S1 and S2) to facilitate the comparison with the results from the previous irrigation experiments at Leytron with the same vine varieties (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018). Soil water potential has been shown to be well correlated with Ψ_{pd} (Bréda et al., 1995) only when the soil water conditions are homogeneous (Améglio et al., 1999). As the soil water conditions are homogeneous at Leytron vineyards, we could safely use Ψ_{pd} or Ψ_{stem} as a reasonable proxy for soil water availability.

The N status of the vines was assessed in the vineyard by measuring the leaf chlorophyll index (LCI) (Spring and Zufferey, 2000) and in the grape berries by measuring the YAN. The LCI values were used to monitor the leaf chlorophyll content at veraison. For this purpose, the LCI was measured every three weeks in 30 mature primary leaves randomly selected in the medial part of the canopy from the 10 vines from each replicate block per treatment using an N-Tester chlorophyll meter (Yara, Paris, France). The instrument provided the average LCI measurements per block, providing four replicates for each treatment. The grapes from the four replicate blocks per treatment were harvested separately. The YAN was determined in aliquots of berry juice from each block (i.e., four replicates per treatment) using near-infrared spectroscopy (FOSS WineScan® instrument, FOSS, Hillerød,

Denmark) at the oenological laboratory at Agroscope Changins, Switzerland. The YAN content is the total N fraction (expressed in mg N L⁻¹) in grape juice available to yeast for alcoholic fermentation. The YAN comprises ammonium ions (NH₄⁺) and free amino acids (AAs), except for proline and hydroxyproline.

2.3. Wine making, wine solid residues and C and N isotope analysis

Grape yield (kg m⁻²) was determined by individually harvesting the vines per treatment block. The grapes of the four treatment replicates were pooled for a single vinification per treatment. The sugars were separated and purified from must aliquots in solution using Dowex 50 V/X8 cation exchange resin (Fluka, Buchs, Switzerland) and the anion exchange resin Merck III (Merck, Darmstadt, Germany) in the laboratories of Agroscope. The $\delta^{13}\text{C}_{\text{sugars}}$ values were determined at the stable isotope laboratories of the Institute of Earth Surface Dynamics of the University of Lausanne (IDYST-UNIL). The $\delta^{13}\text{C}$ value of the sugar fraction of the juice of mature berries (shortly before harvest) integrates the photosynthetic C isotope fractionation during the entire period between bloom and harvest (Gaudillère et al., 2002). The sugars in the berry juices or musts may contain some contribution from the remobilized root carbohydrate reserves from previous seasons; their effect on the $\delta^{13}\text{C}_{\text{sugars}}$ of berry juice is most likely minimal (Spangenberg and Zufferey, 2018). Wines were prepared for the single treatments, for each variety and season, using the same yeast strains (*Saccharomyces cerevisiae*) and vinification protocol at the Agroscope research winery (Changins-Wädenswil, Switzerland). The vinification procedure is described in detail in Supplementary Text S1. Here, it is important to note that no supplementary N nutrients for yeast were added to the must. There was only one difference in the production of red and white wines. The making of white wines (Chasselas and Petite Arvine) was performed by alcoholic fermentation of the grape juices without skin; for making red wine (Pinot noir), the crushed complete grape (with exocarp) was fermented. The exocarp contains most of the grape N (mainly in proteins and breakdown products) and promotes fermentation with the indigenous microflora. Four wines were produced per grape variety and season, corresponding to the four treatments (BS-DI, BS-NI, GS-DI, and GS-NI). Two bottles of each wine were brought to the IDYST-UNIL laboratories and stored in the dark at 4 °C until use.

The solid wine residues were obtained by freeze-drying aliquots of wine. The freeze-drying process was performed in duplicate with wine aliquots from separate bottles. For each wine bottle, a subsample of 25 mL was transferred to 50 mL LDPE bottles (wide-necked bottles with screw caps, VWR International AG, Dietikon, Switzerland) and frozen at -20 °C for two days before being freeze-dried on a Lyovac GT2 freeze dryer (SRK Systemtechnik GmbH, Goddelau, Germany) for 48 h. The freeze-dried wines were stored at -20 °C until isotopic analyses, which were performed in duplicate and repeated one or, occasionally, two times. The C isotope composition of must sugars and the C and N isotope compositions of WSR (four to eight analytical replicates each, see below) were determined for each treatment (BS-DI, BS-NI, GS-DI, and GS-NI), variety, and season (2020, 2021).

The C and N isotope compositions were determined by continuous helium (He) flow elemental analysis/isotope ratio mass spectrometry (EA/IRMS) using an elemental analyzer (Carlo Erba 1108, Fisons Instruments, Milan, Italy) connected to an isotope ratio mass spectrometer via a split interface (Delta V Plus and ConFlo III, both from Thermo Fisher Scientific, Bremen, Germany). The C and N contents and isotope ratios were determined by separated combustions using different sets of dedicated calibration standards and sample aliquots of different weight sizes. The isotopic compositions were reported in the delta (δ) notation corresponding to the relative deviations of the molar ratio (R) of the heavy (¹³E, ¹⁵N) to light (¹²E, ¹⁴N) isotopes in the samples from that in international standards:

$$\delta^i E_{\text{sample}} = \frac{R^{(hE/lE)}_{\text{sample}}}{R^{(hE/lE)}_{\text{standard}}} \quad (1)$$

For $\delta^{13}\text{C}$ values, the standard is Vienna Pee Dee Belemnite limestone (VPDB); for $\delta^{15}\text{N}$ values, the standard is Air-N₂ (molecular N in air). The International System of Units (SI) unit for the delta values is the urey (symbolized Ur), according to the guidelines and recommendations of the International Union of Pure and Applied Chemistry (IUPAC)—Commission on Isotopic Abundances and Atomic Weights (Brand and Coplen, 2012). The δ -values from the above equation were multiplied by 1000, and the unit is milliurey (mUr), synonym of the deprecated per mil (‰), which is not an SI unit. The isotopic ratios were normalized using a three-point calibration with in-house and international reference materials. The repeatability and intermediate precision of the EA/IRMS $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements were determined by the standard deviation of separately replicated analyses ($n = 4$ to 6) and were better than ± 0.1 mUr. The total organic C and N contents (wt% TOC and TN, respectively) were determined from the sum of the major peak areas. The repeatability was better than 0.2 wt% for C and N concentrations. Further details on the EA/IRMS analysis and the standards used for the calibration and normalization of measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be found in Supplementary Text S1.

The field and laboratory measurements are presented in a graphical (plot layout, symbol type, and colors) and statistical style similar to the published results for irrigation experiments with the same vine varieties at the same experimental station in Leytron (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018) to allow comparison and reassessments. Data were compiled using Microsoft Excel V16.65 (Microsoft Corporation, Redmond, WA, USA) and SPSS V20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The results are reported as the mean \pm standard error (SE) from for biological replicates per treatment for Ψ_{stem} , LCI, YAN content, and yield and from analytical replicates per treatment for $\delta^{13}\text{C}_{\text{sugars}}$ ($n = 6$), $\delta^{13}\text{C}_{\text{WSR}}$ ($n = 4-6$), and $\delta^{15}\text{N}_{\text{WSR}}$ ($n = 8$). The data were tested for homogeneity of variance (F test), and the comparisons between the means of each group were performed by paired-samples

Student's t -tests with the significance level set at $P < 0.05$. Graphics were prepared using DeltaGraph V7.1.3 (Red Rock Software Inc., Salt Lake City, UT, USA), Adobe Illustrator 2022 V27.1.1 (Adobe Systems Inc., CA, USA), and 2022 Microsoft PowerPoint V16.69.1. Pearson correlation coefficients and linear regression lines were calculated with Microsoft Excel V16.65 and DeltaGraph V7.1.3.

3. Results and discussion

3.1. Vine water status

The Ψ_{stem} values of the Chasselas, Petite Arvine, and Pinot noir vines from the different soil management and irrigation treatments are presented in Fig. 1 and Supplementary Tables S1 and S2. The changes in the plant water status were determined primarily from the soil water availability established by the soil water treatments (DI, NI), and in the case of rainfed vines, combined with the dryness of the vintage summer (hot and dry in 2020, humid and warm in 2021). The lowest Ψ_{stem} values were measured in 2020 grapevines grown on grass-covered and nonirrigated soils (-1.16 ± 0.05 MPa for Pinot noir, -1.38 ± 0.04 MPa for Chasselas, and -1.43 ± 0.06 MPa for Petite Arvine; Table S2). The high water-stressed plants (i.e., with Ψ_{stem} values lower than -1.1 MPa, van Leeuwen et al., 2009) were the nonirrigated Chasselas and Petite Arvine vines for both 2020 treatments (BS-NI, GS-NI; Ψ_{stem} values between -1.43 and -1.22 MPa) and Pinot noir BS-NI vines of 2020 (-1.16 MPa). The only nonwater-stressed vines (i.e., with Ψ_{stem} values higher than -0.6 MPa) were the Pinot noir (-0.49 ± 0.03 MPa), Petite Arvine (-0.55 ± 0.03 MPa), and Chasselas (-0.61 ± 0.03 MPa) vines on rainfed bare soils in 2021.

The $\delta^{13}\text{C}_{\text{sugar}}$ values of all must samples (grape varieties, soil management, irrigation treatments, 2020 and 2021 seasons) varied between

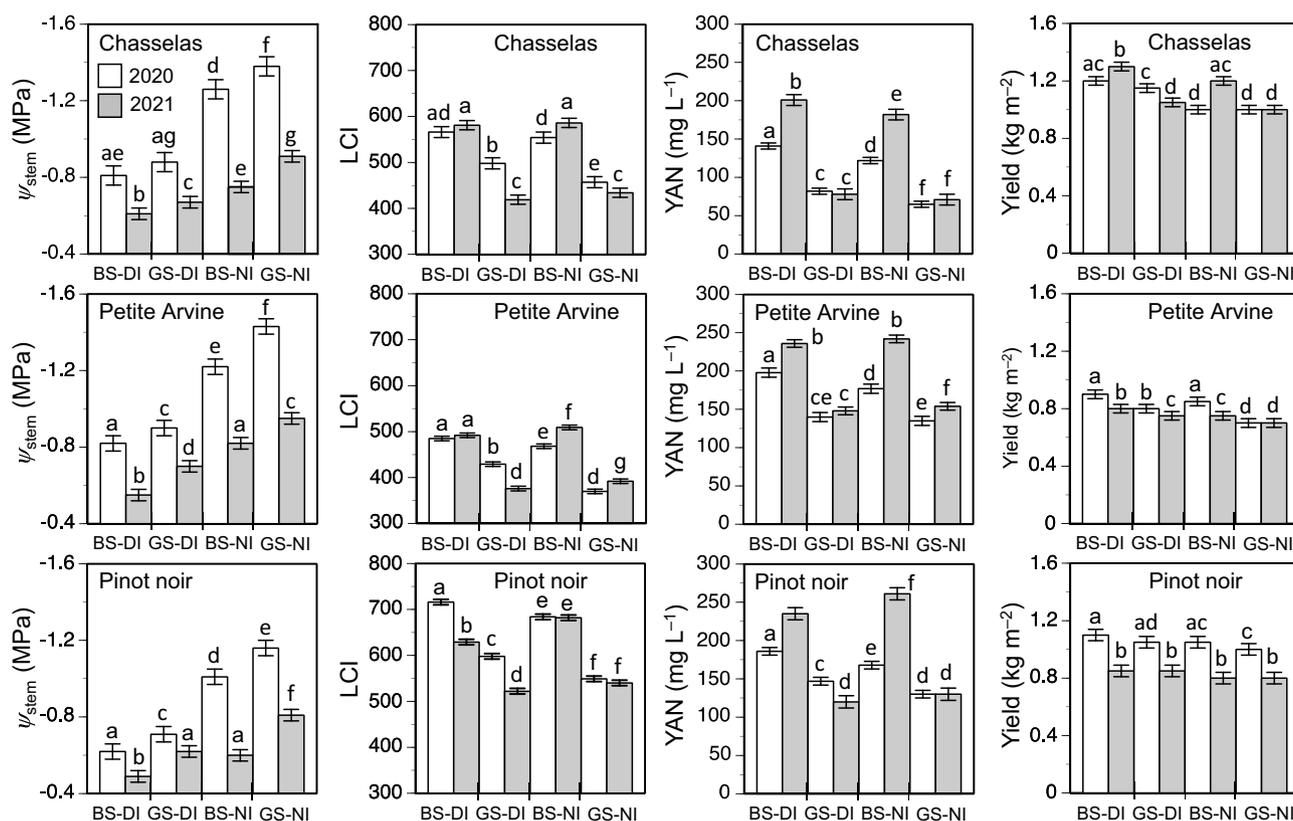


Fig. 1. Physiological and agronomic parameters of Chasselas, Petite Arvine, and Pinot noir vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during the 2020 and 2021 growing seasons. The error bars represent the standard error (SE) of the mean from four biological replicates for the stem water potential (Ψ_{stem}), leaf chlorophyll index (LCI), yeast assimilable nitrogen (YAN) in must, and crop yield. Columns with different lower-case letters are significantly different at $P < 0.05$ according to Student's t -test.

–27.43 and –23.76 mUr (–26.19 ± 1.05 mUr) (Fig. 2, Tables S1 and S2). This 3.7 mUr wide range of $\delta^{13}\text{C}_{\text{sugars}}$ values shows the strong impact of water availability on the C isotope composition of plant products. The relationship between vine water status and the C isotope composition of must sugars in vineyards was first reported by Gaudillère et al. (2002). We previously used this link to trace the water deficit in irrigation experiments at Leytron (Switzerland) with Chasselas, Petite Arvine, and Pinot noir during the 2009 to 2016 growing seasons (Zufferey et al., 2017, 2018, 2020). The $\delta^{13}\text{C}_{\text{sugars}}$ and Ψ_{stem} values were highly correlated for all grapevines (Fig. 3A, Pearson correlation coefficient, $r = -0.86$, $P < 0.00001$, $n = 24$) and for the individual vine varieties (Fig. 3B–D, with all having the same $r = -0.91$, $P < 0.002$, $n = 8$). However, there were differences in the $\delta^{13}\text{C}_{\text{sugars}}-\Psi_{\text{stem}}$ relationship between the white and red grapevine varieties (Figs. 3B–D and Table S2). For Chasselas and Petite Arvine, the $\Delta^{13}\text{C}_{\text{sugars}}$ values varied between –27.0 and –23.8 mUr, covering a range (abbreviated as $\Delta^{13}\text{C}_{\text{sugars}}$) of 3.27 and 3.63 mUr, respectively. These shifts in $\delta^{13}\text{C}_{\text{sugars}}$ corresponded to changes in the Ψ_{stem} values (abbreviated as $\Delta\Psi_{\text{stem}}$) of 0.77 MPa for Chasselas and 0.88 MPa for Petite Arvine. In Pinot noir vines, the shifts in the $\delta^{13}\text{C}_{\text{sugars}}$ and Ψ_{stem} values were smaller: 2.74 mUr for $\Delta^{13}\text{C}_{\text{sugars}}$ and 0.67 MPa for $\Delta\Psi_{\text{stem}}$. The differences per variety in the $\Delta^{13}\text{C}_{\text{sugars}}$ and $\Delta\Psi_{\text{stem}}$ values suggest that Chasselas and Petite Arvine vines responded to changes in the undervine soil treatments and soil water supply conditions to a similar extent. These responses were more pronounced than those of Pinot noir grapevines. This observation may be related to the high environmental metabolic and phenotypic plasticity of Pinot noir leaves (Castagna et al., 2017). In this context, we recently showed a higher rate of epicuticular wax accumulation on Pinot noir leaves than on Chasselas leaves in response to plant water deficit (Spangenberg et al., 2020). The changes in the concentrations and $\delta^{13}\text{C}$

values of the leaf-surface lipids provided evidence for higher plasticity and environmental adaptability in Pinot noir than in Chasselas.

3.2. Vine nitrogen status

The LCI values at veraison and the YAN content in grape juice are depicted in Fig. 1 and listed in Tables S1 and S2. The LCI values varied between 370 and 716 for all treatments and vine varieties. All Pinot noir vines had relatively high LCI values (522–727, 615 ± 74) compared to the white grapevine varieties, and the highest values were measured in well-watered grapevines on bare soils during 2020 (Table S2). In Chasselas and Petite Arvine, the higher LCI values were measured in rainfed vines on bare soils in the 2021 season. Lower LCI values were measured in vines on grass-covered grounds in both seasons (Figs. 1 and S1). The LCI estimates the N supply, uptake, and physiological development of the plant and, as such, provides an indication of potential vine N deficiencies (Nacry et al., 2013). In water-deficient soils, soluble N is limited, strongly restricting the plant uptake of N. The vine-weed competition for water and water-soluble N further hampers the dynamics and vine N uptake from grass-covered soils. Nitrogen deficiency in the GS-NI vines was evidenced in the field by changes in leaf color and lower plant vigor. The leaf color change generally started after veraison in young leaves and shoot tips, and the margin of mature leaves changed to pale green and yellowish green. The progressive changes in leaf color, vine vigor, and chlorophyll content during the 2020 season suggested that the nonirrigated vines on grass-covered soils were under water- and N-stress compared to the well-watered vines on bare soils. These stresses resulted from a dynamic competition for water, nutrients, and growth between the shallow-rooted grasses (<15 cm) and grapevines. These results are in line with the findings of a

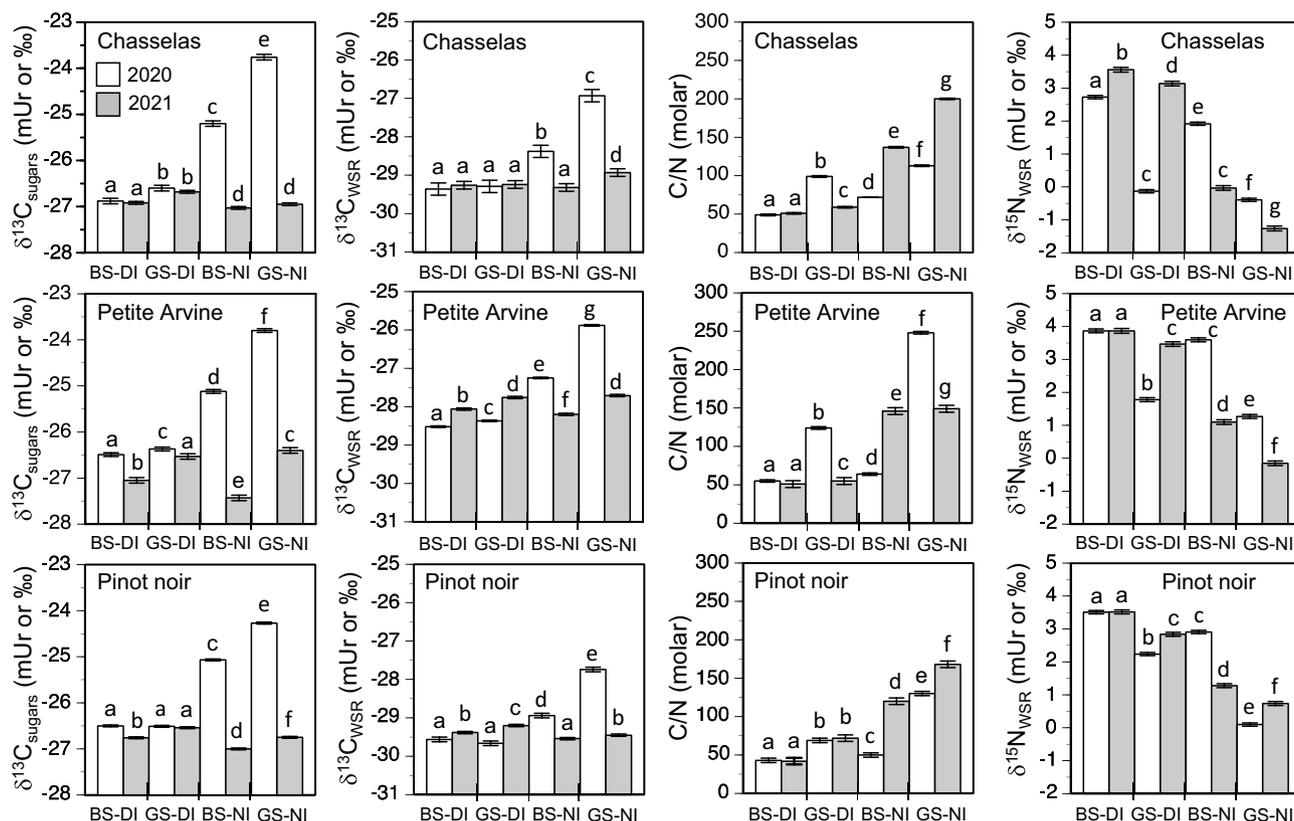


Fig. 2. Carbon and nitrogen contents and isotope ratios of products from Chasselas, Petite Arvine, and Pinot noir vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021. The error bars represent the standard error (SE) of the mean from replicate determinations of the carbon isotope composition of must sugars ($\delta^{13}\text{C}_{\text{sugars}}$, $n = 6$) and wine solid residue ($\delta^{13}\text{C}_{\text{WSR}}$, $n = 4-6$), C/N molar ratio ($n = 4-6$), and nitrogen isotope composition of wine solid residue ($\delta^{15}\text{N}_{\text{WSR}}$, $n = 8$). Columns with different lower-case letters are significantly different at $P < 0.05$ according to Student's *t*-test.

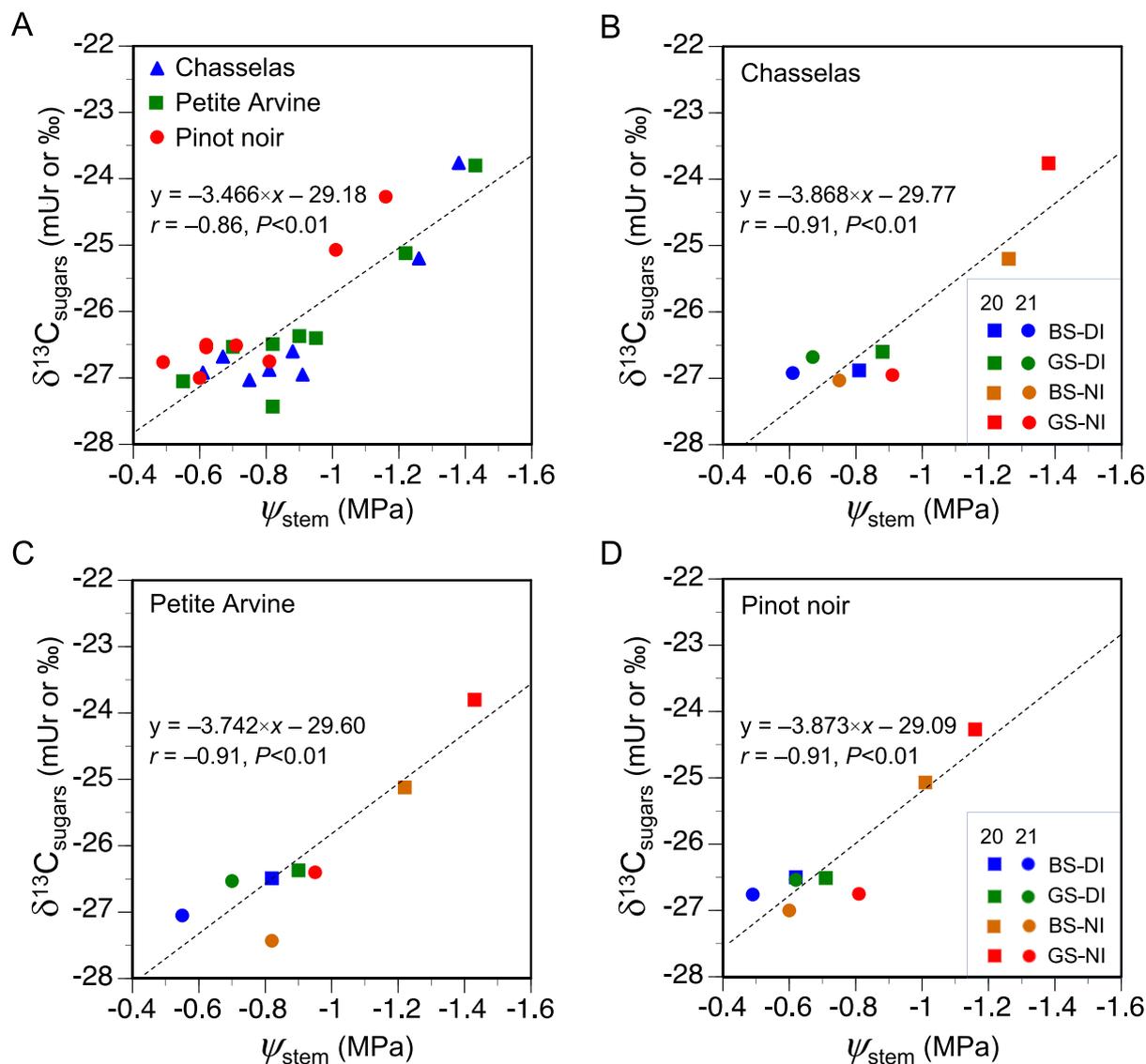


Fig. 3. Carbon isotope composition of must sugars ($\delta^{13}\text{C}_{\text{sugars}}$) versus the stem water potential (Ψ_{stem}) of all vines (A) and the Chasselas (B), Petite Arvine (C), and Pinot noir (D) vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021.

previous study, showing that cover crop in an unfertilized vineyard under the Mediterranean climate triggered competition for soil N (Celette et al., 2009).

The YAN content in musts provided further insight into the extent of the N deficiency. For all vines, the YAN content varied between 65 and 251 mg L^{-1} (Fig. 1 and Tables S1 and S2). At the variety level, the Petite Arvine musts had YAN levels ($178.8 \pm 42.5 \text{ mg L}^{-1}$) similar to those of Pinot noir ($172.1 \pm 52.0 \text{ mg L}^{-1}$) and higher (statistically significant for plants grown under the same conditions, Fig. 1) than those of Chasselas ($156.2 \pm 54.8 \text{ mg L}^{-1}$). The correlation coefficients between the YAN and Ψ_{stem} values were significant at $P < 0.05$ for all vines ($r = 0.48$, $P = 0.017$, $n = 24$) but not for the individual vine varieties (Supplementary Fig. S2). Among all varieties, the highest YAN levels were measured in the vines of the BS-DI treatment in both seasons (Fig. 1). For Pinot noir and Petite Arvine, the YAN contents were higher in the BS-NI treatment during the warm and humid season of 2021. All treatments with grass-covered soil, regardless of the soil-water treatment (well-watered or rainfed) and season climate, had similar low YAN levels (Figs. 1 and S2B-D). These observations suggest that YAN concentrations decrease more severely because of vine/cover-crop competition for N rather than as an effect of restriction of the uptake of water-soluble N in water-deficient rainfed

vines on bare soil (e.g., BS-DI vs. GS-DI and BS-DI vs. BS-NI in Figs. 1, S2B-D). This result is important because it shows that weeds compete for N even if the soil is well irrigated and contains water-soluble N. This statement is valid for the studied vine cultivars (Chasselas, Petite Arvine, and Pinot noir) and the cover cropping MCS4 mixture.

Finally, a minimal amount (140 mg N L^{-1}) of assimilable amino-N is required to ensure the completion of the alcoholic fermentation of grape juices and obtain high-quality wine (Jiranek et al., 1995; Garde-Cerdan and Ancin-Azpilicueta, 2008; Garde-Cerdán et al., 2014). Clear musts of white grapes with YAN $< 140 \text{ mg L}^{-1}$ and an average sugar concentration have a high risk of stuck and sluggish alcoholic fermentation (Bisson and Butzke, 2000; Bell and Henschke, 2005). In musts of red grapes, the YAN threshold is lower due to the longer contact of the berry skin with grape juice, allowing microorganisms to degrade leaf proteins, releasing N compounds (e.g., amino acids) that contribute to the total YAN content. It was recently shown that the minimum YAN levels required for the successful fermentation of Pinot noir musts were as low as 100 mg L^{-1} (Schreiner et al., 2018). These minimal limits for YAN may be underestimated for must with high concentrations of sugars (Childs et al., 2015). High-sugar musts are being obtained more frequently in the context of ongoing climate change. All of the musts from the Chasselas vines on grass-covered soils had

very low YAN content (71–83 mg L⁻¹). Such low levels of YAN in must would require a relatively high N supplementation for complete fermentation. For Petite Arvine, the musts from the ground grass-covered vines from the hot and dry 2020 had low and marginal YAN levels for both soil water treatments (140 and 135 mg L⁻¹, for DI and NI, respectively). All of the Pinot noir grape musts had YAN > 120 mg L⁻¹, and the values were relatively low for the cover crop treatments (Figs. 1 and S2). Moderate correlations were observed between YAN and LCI ($r = 0.47, P = 0.020, n = 24$) for all vines (Table S1). However, high YAN-LCI correlations (significant at $P < 0.005$) were observed in the white grape varieties ($r = 0.90$ with $P = 0.002$ for Chasselas, $r = 0.89$ with $P = 0.003$ for Petite Arvine) and were acceptable ($P < 0.05$) for Pinot noir ($r = 0.71$ with $P = 0.049$) (Fig. S3, Table S2). The differences in the YAN-LCI correlations for all vines and per-cultivar vines may be explained by differences in N uptake and N metabolism for the studied cultivars/genotypes. The YAN of Pinot noir musts may contain soluble amino acids and proteins released from grape berry skin during maceration (e.g., degradation of cell wall proteins) (Aron and Kennedy, 2007; Zietsman et al., 2015). These results are in line with a

reported cultivar-specificity of the YAN concentrations, which is stronger in red than in white grapevine cultivars (Petrovic et al., 2019).

For Chasselas, no clear differences in crop yield were observed between the 2020 and 2021 seasons and due to the combined cover-crop and irrigation treatments (Fig. 1, Tables S1 and S2). For Petite Arvine and Pinot Noir, the crop yields were higher in 2020 compared to 2021 for all treatments.

3.3. Carbon isotope composition of wine solid residues

The WSRs had total organic C (TOC_{WSR}) contents between 30.3 and 35.3 wt% (32.8 ± 1.3 wt%), with C isotope compositions ($\delta^{13}\text{C}_{\text{WSR}}$) of -29.66 to -25.88 mUr (-28.58 ± 0.98 mUr) (Fig. 2 and Tables S1 and S2). The differences between the $\delta^{13}\text{C}_{\text{sugars}}$ and $\delta^{13}\text{C}_{\text{WSR}}$ values ($\Delta^{13}\text{C}_{\text{sugars-WSR}} = \delta^{13}\text{C}_{\text{sugars}} - \delta^{13}\text{C}_{\text{WSR}}$) for all wine varieties were on average 2.39 ± 0.77 mUr (2.59 ± 0.42 mUr for Chasselas, 1.57 ± 0.55 mUr for Petite Arvine, and 3.01 ± 0.45 mUr for Pinot noir; Table S2). These differences match the C isotope fractionation of 2 mUr associated with the fermentation of C₃-derived glucose to ethanol (Hobbie and Werner, 2004).

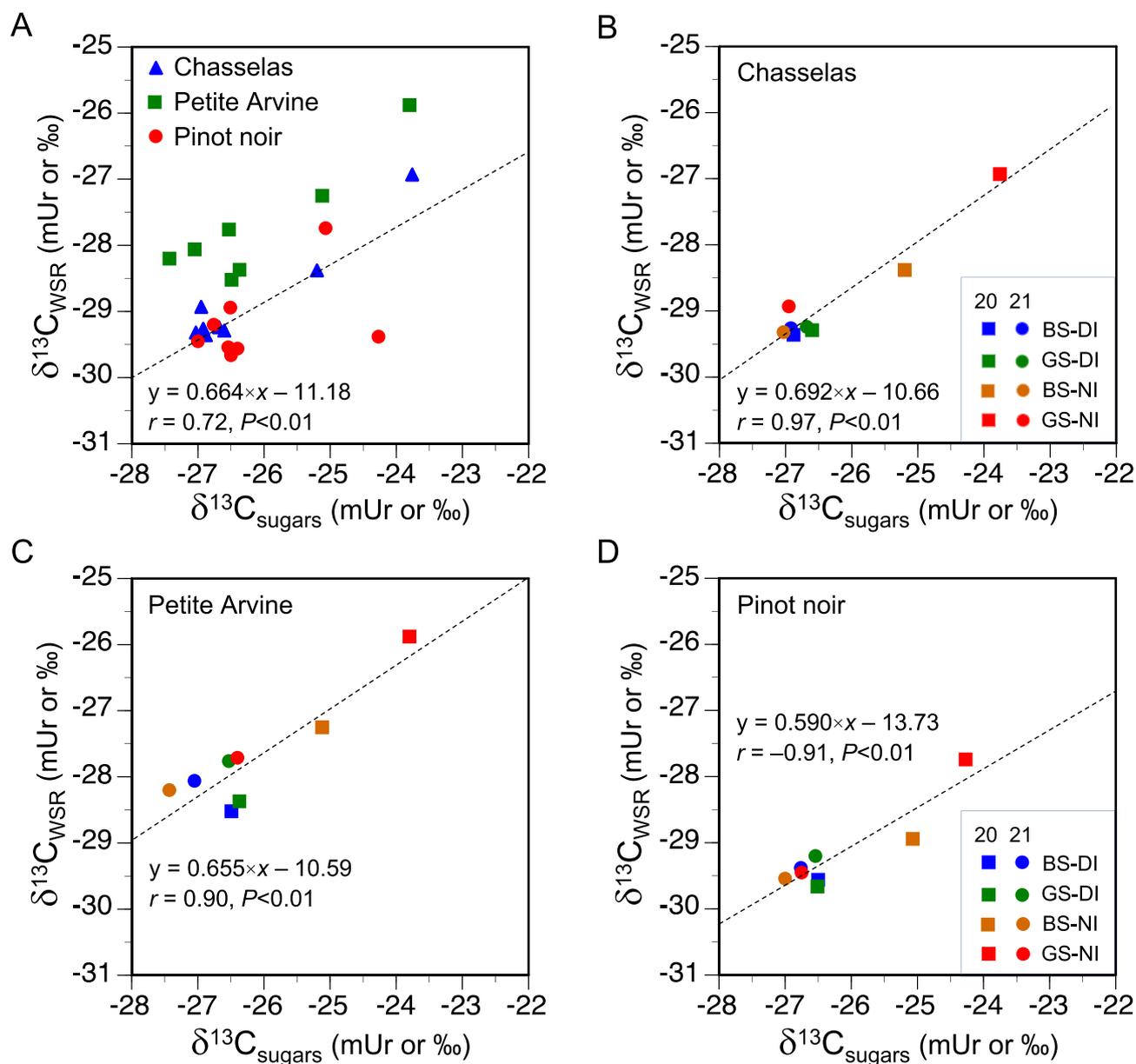


Fig. 4. Carbon isotope composition of wine solid residue ($\delta^{13}\text{C}_{\text{WSR}}$) versus the must sugars ($\delta^{13}\text{C}_{\text{sugars}}$) of all vines (A) and the Chasselas (B), Petite Arvine (C), and Pinot noir (D) vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021.

The higher $\Delta^{13}\text{C}_{\text{sugars-WSR}}$ values for Pinot noir were due to the relatively lower $\delta^{13}\text{C}_{\text{WSR}}$ values compared to those of Chasselas and Petite Arvine. The relatively lower $\delta^{13}\text{C}_{\text{WSR}}$ of Pinot noir wines can be explained by the contribution of ^{13}C -depleted compounds (e.g., lipids such as fatty acids and esters) released from grape skin in must during maceration and alcoholic fermentation. Lower $\delta^{13}\text{C}_{\text{WSR}}$ values were measured in Pinot noir wines derived from drip-irrigated GS and BS vines in the 2020 season and rainfed BS vines in the 2021 season (Fig. 4). The highest $\delta^{13}\text{C}_{\text{WSR}}$ values were in Petite Arvine wines from the 2020 rainfed BS and GC vines. The $\delta^{13}\text{C}_{\text{WSR}}$ values were highly correlated with $\delta^{13}\text{C}_{\text{sugars}}$ ($r = 0.71$, $P = 0.0001$, $n = 24$) for the three cultivars (Fig. 4). The high correlation between the $\delta^{13}\text{C}_{\text{sugar}}$ and the degree of plant water deficit ($r = -0.86$ for $\delta^{13}\text{C}_{\text{sugars-}\Psi_{\text{stem}}}$, $P < 0.00001$, $n = 24$; Fig. 3) was well preserved in the WSRs ($r = 0.75$ for $\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{stem}}}$, $P < 0.0001$, $n = 24$) (Fig. 5). The $\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{stem}}}$ relationships exhibited similar regression lines for the three vine varieties. These $\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{stem}}}$ relationships mimic the $\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{pd}}}$ relationships reported for the same white and red wine varieties from the 2009–2014 irrigation experiments on bare soil (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018). The 2020 and 2021 results confirm that the linear relationship between the $\delta^{13}\text{C}$ values of the solid residue from freeze-dried wines and a measure of the degree of plant water stress

($\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{pd}}}$ or $\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{stem}}}$) is most likely valid for all varieties. However, notably, the intercepts of the $\delta^{13}\text{C}_{\text{WSR-}\Psi_{\text{pd/stem}}}$ regression equations may change. The Petite Arvine WSRs from the 2009–2015 irrigation experiments were systematically ^{13}C -enriched by ca. +1.1 mUr compared to those of Chasselas and Pinot noir (Spangenberg and Zufferey, 2018). These differences between cultivars were confirmed in the 2020–2021 soil management trials. The $\delta^{13}\text{C}_{\text{WSR}}$ values for Petite Arvine were consistently higher than those for the other varieties, i.e., $+1.12 \pm 0.20$ mUr for Chasselas and $+1.47 \pm 0.28$ mUr for Pinot noir (Table S2). Further studies using the same approach in other cultivars and environmental settings (i.e., climate, soil type) will permit the exclusion of a cultivar effect on the changes in the $\delta^{13}\text{C}_{\text{WSR}}$ values due to the vine water stress level.

3.4. C/N molar ratios and N isotope ratios of wine solid residues

The TN_{WSR} values for all wine samples ranged from 0.15 to 0.94 wt%, without significant differences among the varieties (0.48 ± 0.21 wt% for Chasselas, 0.46 ± 0.25 wt% for Petite Arvine, 0.58 ± 0.28 wt% for Pinot noir) (Tables S1 and S2). The difference between the TN_{WSR} values of both types of soil management ($\Delta\text{TN}_{\text{GS-BS}}$) for the different irrigation treatments (DI, NI), seasons (20,2021), and wine varieties (Chasselas, Petite

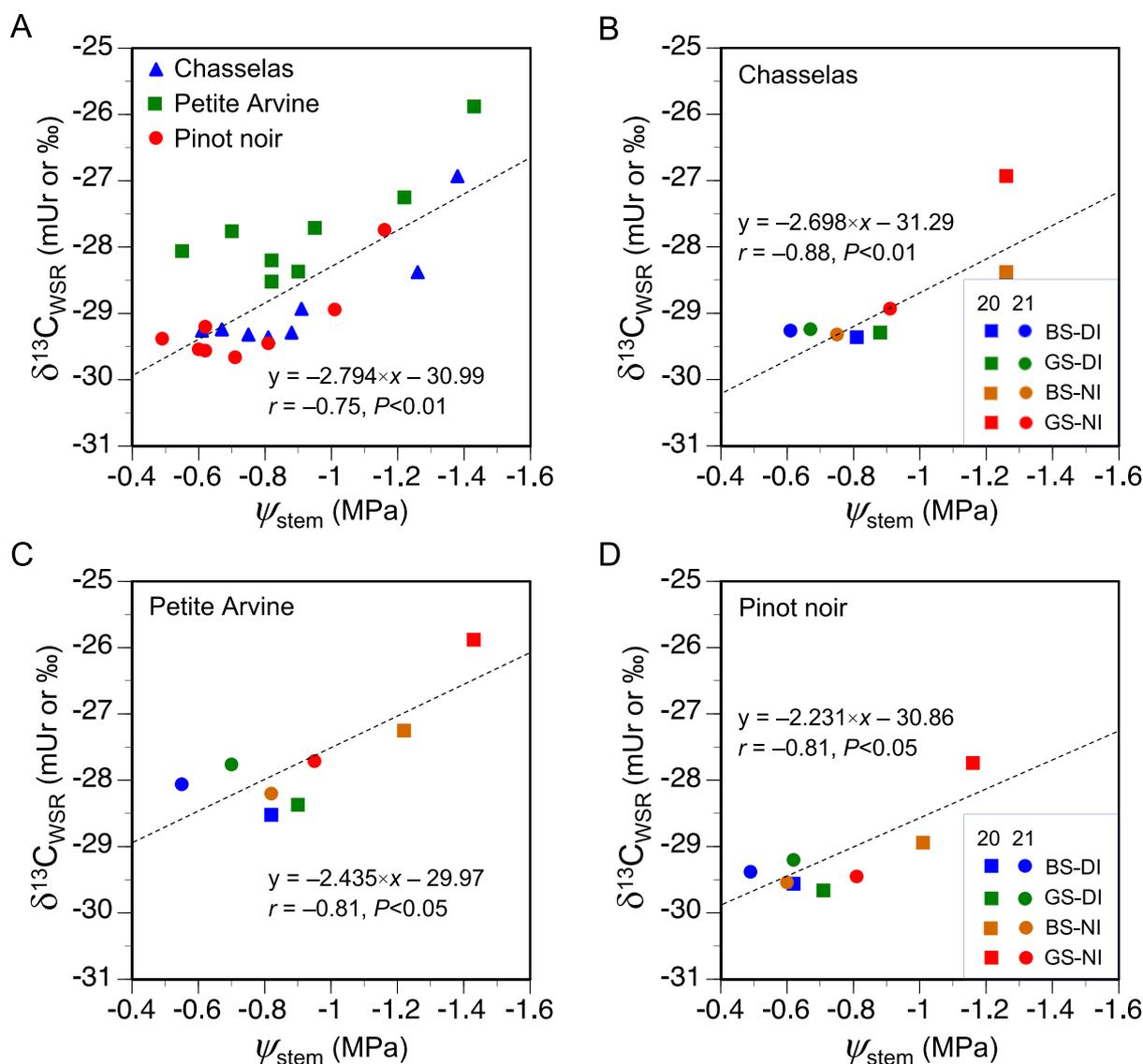


Fig. 5. Carbon isotope composition of wine solid residue ($\delta^{13}\text{C}_{\text{WSR}}$) versus the stem water potential (Ψ_{stem}) of all vines (A) and the Chasselas (B), Petite Arvine (C), and Pinot noir (D) vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021.

Arvine, and Pinot noir) varied from -0.51 to -0.03 wt% (Table S1, Fig. S4). Lower TN contents were measured in wines on grass-covered soils, and the levels were even lower in wines from rainfed vines of the hot and dry 2020 summer. These trends were clearly seen in Pinot noir and Chasselas and not in Petite Arvine, which showed atypical behavior similar to that reported for the 2009–2015 irrigation experiments (Spangenberg and Zufferey, 2018). Further details on the variation in the N content in wines with soil management can be seen in the bar plots of the C/N_{WSR} molar ratios and the $C/N_{WSR}-\Psi_{stem}$ scatterplots (Figs. 2 and 6). There was a clear separation between the BS vs. GS and DI vs. NI wines in the $C/N_{WSR}-\Psi_{stem}$ space for Chasselas and Pinot noir (Fig. 6B and D), comparable to that observed for vines in the LCI- Ψ_{stem} and YAN- Ψ_{stem} plots (Figs. S1 and S2). The C/N_{WSR} ratios increased (lower TN content) in the order BS-DI < GS-DI < BS-NI < GS-NI for Chasselas and Pinot noir (Fig. 6B and D). Again, for Petite Arvine, the $C/N_{WSR}-\Psi_{stem}$ relationships were not clear. The extreme N deficit in the GS-NI wines was due to the combined effect of vine-weed competition for N and the restriction of N uptake by roots in water-deficient soils. The effect of immobilization of soil N by microbial biomass, which would be expected to be greater in grass-covered soils than bare soils, cannot be excluded (Steenwerth and Belina, 2008). On the other hand, wines from well-watered vines on grass-covered soil had similar

(i.e., Chasselas and Petite Arvine from 2021) or slightly lower (Chasselas and Petite Arvine from 2020 and Pinot noir wines for both seasons) N contents than the wines from drip irrigation treatments.

The N isotope compositions of the WSRs showed clear trends with the vine water status (Figs. 2 and 7). The $\delta^{15}N_{WSR}-\Psi_{stem}$ plots (Fig. 7B–D) depicted clear trends that mimicked the level of the N deficit between vines on cover-crop and bare soils observed in the $C/N_{WSR}-\Psi_{stem}$ and $TN_{WSR}-\Psi_{stem}$ relationships for the three varieties (Fig. 6B–D and S4B–D). All of the wines showed a very clear trend toward lower $\delta^{15}N_{WSR}$ values for vines on cover-cropped soil. The difference between the $\delta^{15}N_{WSR}$ values of both types of soil management ($\Delta^{15}N_{GS-BS}$) for the different irrigation treatments, vine varieties, and seasons varied between -2.9 (Pinot noir, GS-BS/NI, hot and dry 2020) and -0.4 mUr (Chasselas and Petite Arvine, GS-BS/DI, warm and humid 2021). The most significant negative $\Delta^{15}N_{GS-BS}$ value (-2.9 mUr) corresponded to the largest ΔTN_{WSR} difference of -0.51 wt%. The depletion in ^{15}N was enhanced by the water deficit, which could be assessed by comparing the $\delta^{15}N_{WSR}$ values of the DI vs. NI conditions for the same soil management (Fig. 7). Notably, the shift in the $\delta^{15}N_{WSR}$ values induced by soil management and the soil water deficit in the dry and hot 2020 season ($\Delta^{15}N_{GS/NI-BS/NI} = -2.9$ mUr) was approximately three times the largest $\delta^{15}N_{WSR}$ shift induced only by water stress

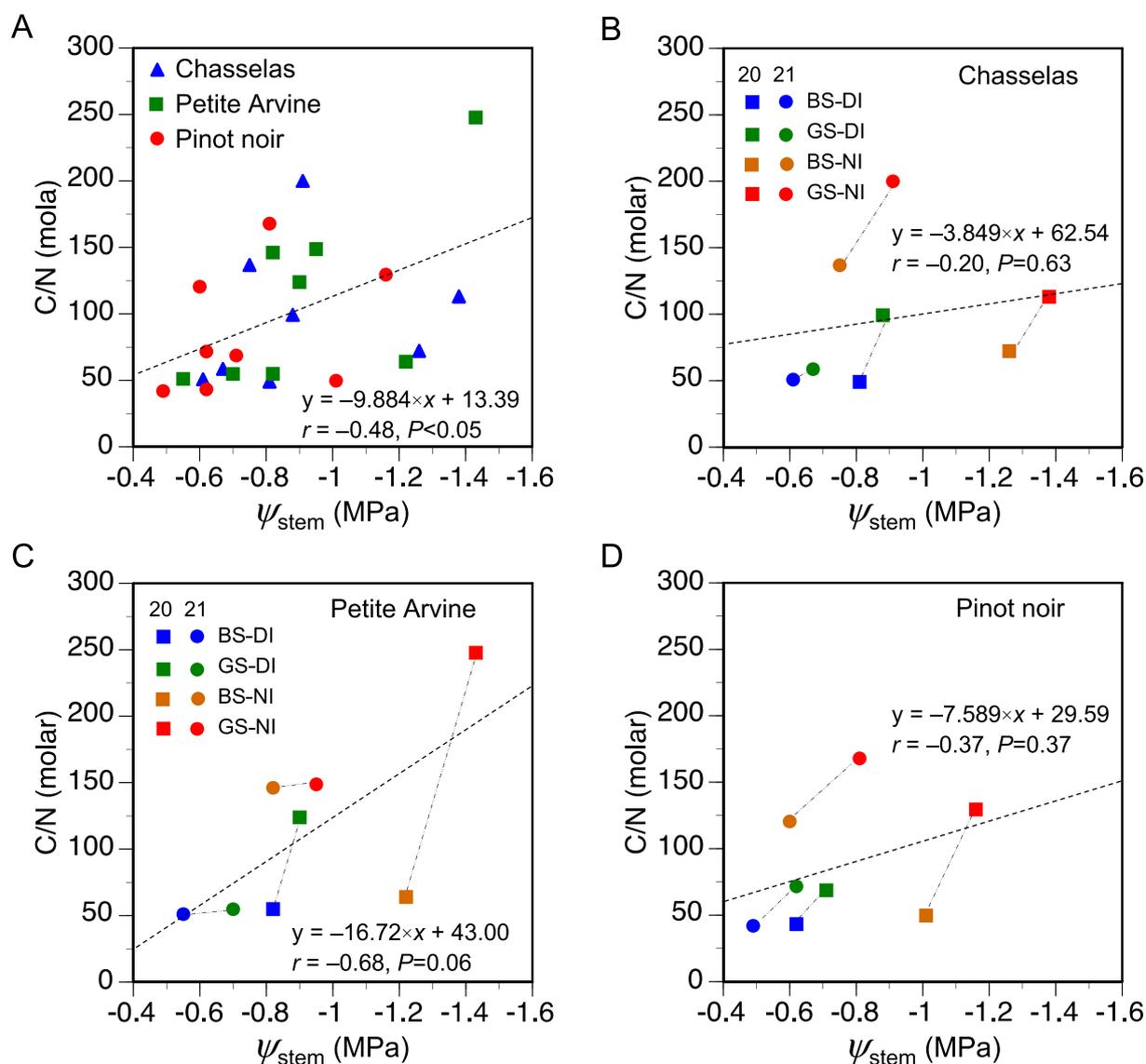


Fig. 6. C/N molar ratio of wine solid residue versus stem water potential (Ψ_{stem}) for all vines (A) and the Chasselas (B), Petite Arvine (C), and Pinot noir (D) vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021. A dashed line joins the two soil management practices for the same irrigation treatment.

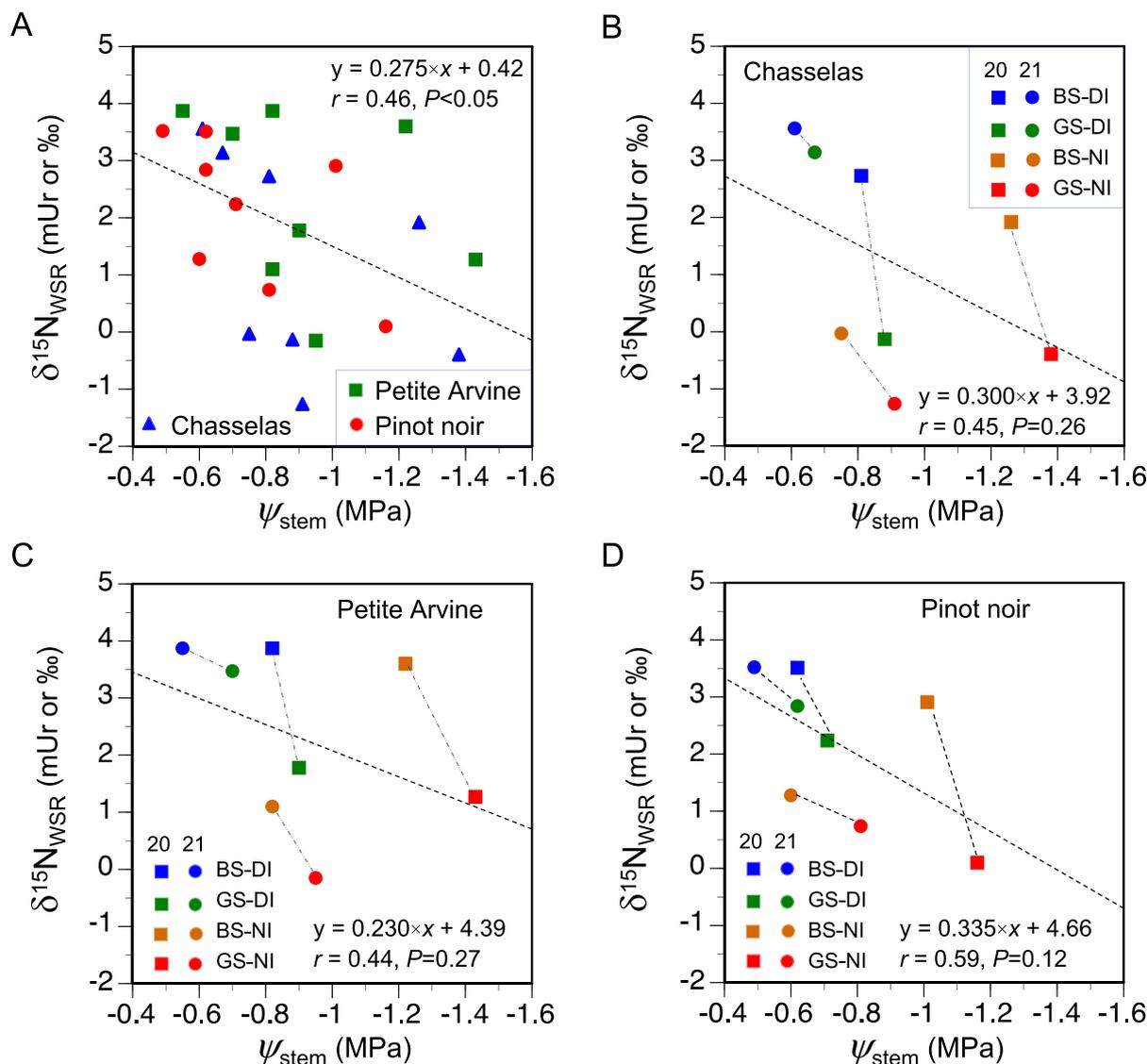


Fig. 7. Nitrogen isotope composition of the wine solid residue ($\delta^{15}\text{N}_{\text{WSR}}$) versus the stem water potential (Ψ_{stem}) of all vines (A) and the Chasselas (B), Petite Arvine (C), and Pinot noir (D) vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021. A dashed line joins the two soil management practices for the same irrigation treatment.

reported for the 2009–2014 irrigation experiments. The maximal effect on the $\delta^{15}\text{N}_{\text{WSR}}$ values induced by water stress in the vines on bare soil was assessed by measuring the difference between the $\delta^{15}\text{N}_{\text{WSR}}$ of wine from nonirrigated and plastic-covered-soil vines and the drip-irrigated vines from the hot and dry 2009, 2010, and 2011 seasons ($\Delta^{15}\text{N}_{\text{NI-NIP}} = -1.2$ mUr) (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018). The N isotope composition and N content (C/N molar ratio or TN content) in the WSRs were highly correlated (Figs. 8 and S5). The lower $\delta^{15}\text{N}_{\text{WSR}}$ values corresponded to the lower TN_{WSR} values in the water-stressed vines (Figs. 6, 7, S4, and S5). This shift mimics that previously observed for the same wines (Chasselas, Petite Arvine, and Pinot noir) from the 2009–2014 irrigation experiment on bare soils. The trend toward lower $\delta^{15}\text{N}$ values in vine products with increasing water deficit indicates the remobilization of organic N (i.e., proteins and their degradation products) from root reserves (Spangenberg and Zufferey, 2018 and references therein). Finally, no statistically significant linear correlations were found in TN_{WSR} vs. YAN and $\delta^{13}\text{C}_{\text{WSR}}$ vs. $\delta^{15}\text{N}_{\text{WSR}}$.

The strong correlations between $\delta^{13}\text{C}_{\text{sugars}}$ and Ψ_{stem} (Fig. 3), $\delta^{13}\text{C}_{\text{WSR}}$ and $\delta^{13}\text{C}_{\text{sugars}}$ (Fig. 4), and $\delta^{13}\text{C}_{\text{WSR}}$ and Ψ_{stem} (Fig. 5) in the grapevines under the two soil surface management conditions and irrigation treatments indicated that the primary photosynthetic C isotope fractionation,

which is affected by the plant water status (assessed by Ψ_{stem} or Ψ_{pd}), was well preserved in the WSRs for the different grapevine varieties (Chasselas, Petite Arvine, and Pinot noir). In this study, the vine water status and the $\delta^{13}\text{C}$ values reflected the soil water availability to plants and, in the case of the GS treatments, the vine competition for water with grasses. Therefore, the $\delta^{13}\text{C}_{\text{sugars}}-\Psi_{\text{stem/pd}}$ and $\delta^{13}\text{C}_{\text{WSR}}-\Psi_{\text{stem/pd}}$ relationships mimicked those found for the same cultivars when different irrigation regimes induced different levels of water deficit in vines growing on weed-free soils during the 2009–2014 seasons (Fig. 9).

The situation is different for nitrogen. The TN_{WSR} content and $\delta^{15}\text{N}_{\text{WSR}}$ value reflect the must N status and source. The concentrations of soluble N species available for uptake by plant roots are very low in water-deficient soils. This N restriction is increased by the efficient competition of weeds for N. The shift toward lower TN values (higher C/N molar ratios) was associated with a decrease in the $\delta^{15}\text{N}_{\text{WSR}}$ values (Figs. 6, 7, and S4) when increasing water deficit and vine-weed competition for N induced a higher contribution of ^{15}N -depleted N stored in roots. The relative importance of these concurrent processes, i.e., the restriction of water-soluble N and vine-and-grass N competition, can be assessed by comparing the C/N- Ψ_{pd} and $\delta^{15}\text{N}_{\text{WSR}}-\Psi_{\text{pd}}$ relationships of the WSRs from the 2020–2021 soil management experiments with those from the 2009–2014 irrigation

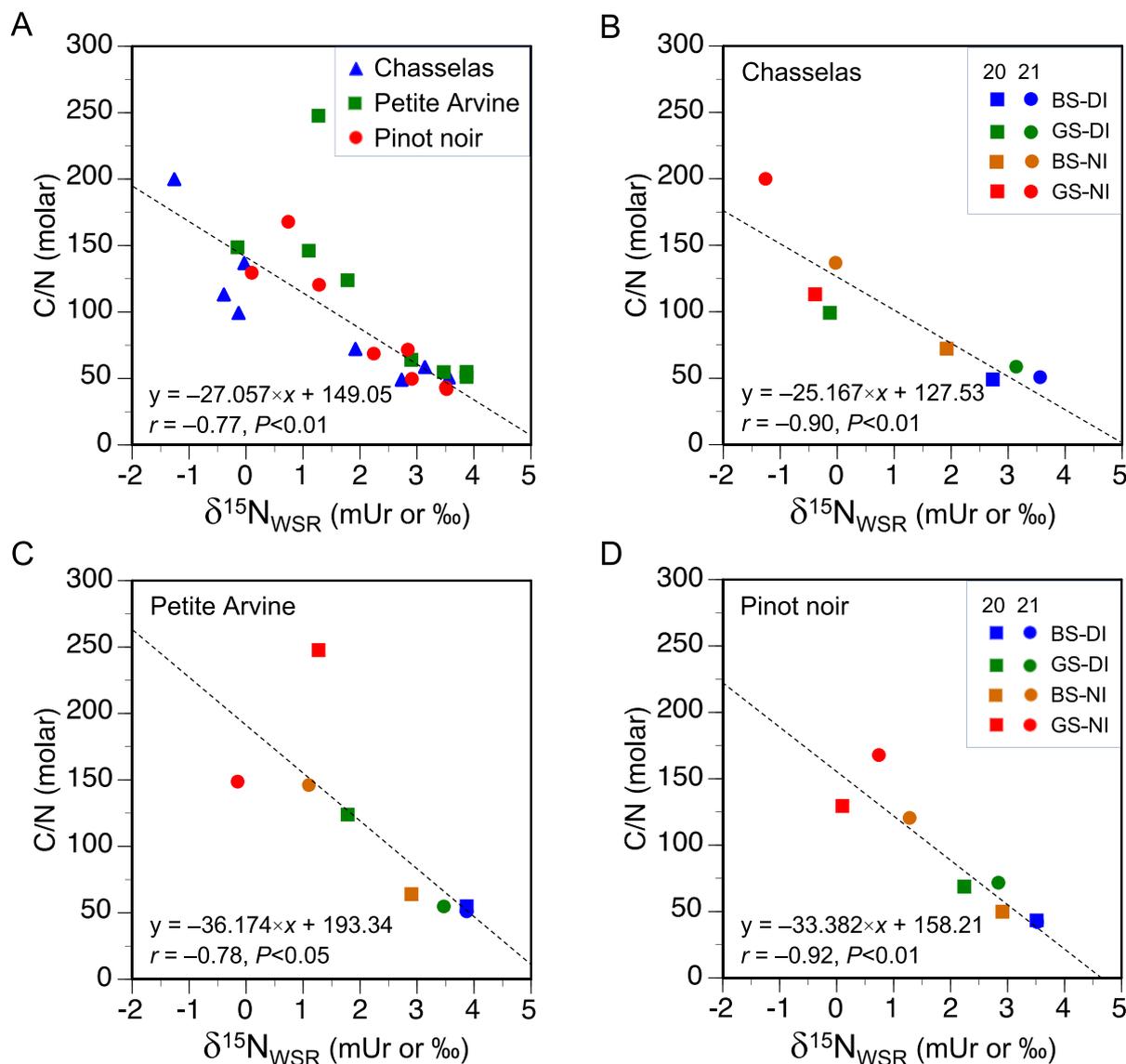


Fig. 8. C/N molar ratio versus the nitrogen isotope composition of the wine solid residue ($\delta^{15}\text{N}_{\text{WSR}}$) of all vines (A) and the Chasselas (B), Petite Arvine (C), and Pinot noir (D) vines grown under different soil management practices (BS = bare soil, GS = grass-covered soil) and water treatments (DI = drip irrigation, NI = no irrigation) during 2020 and 2021.

experiments where all of the vines were on bare ground (Fig. 9C, D). Nine of the 24 2020–2021 samples plot outside the field defined by the 2009–2014 wines. These apparent outliers with higher C/N molar ratios (lower N concentrations) and lower $\delta^{15}\text{N}_{\text{WSR}}$ values corresponded to all of the 2021 non-irrigated vines and the Petite Arvine and Pinot noir vines on rainfed grass-covered soils from the hot and dry 2020 season (see details in Supplementary Figs. S5–S6).

The soil management and irrigation treatments changed the wine characteristics. The Chasselas and Petite Arvine wines from vines that have suffered from water and N stress on nonirrigated grass-covered soil were less appreciated at testing (i.e., loss of typicity, notes of astringency and bitterness) compared to wines from vines on irrigated bare soil. The Pinot noir wines were more colorful and richer in phenolic compounds and had preferred tasting under moderate to high water stress. Cover crops did not significantly affect the olfactory and taste attributes of these red wines. These wine characteristics support the hypothesis that Pinot noir grapevines may adapt better to different soil water conditions and soil management practices than the white grapevines Chasselas and Petite Arvine. We showed, for the three grapevine varieties, that well-watered vines on grass-covered soil produced grapes and wines similar to those obtained by conventional

soil management with herbicides. This result is promising, as it indicates that well-watered vineyards may not need the application of herbicides. However, drip irrigation is not a feasible agroecological solution when entering an era of water scarcity (Postel, 2000).

Some limitations of this study should be acknowledged. First, the study was performed in a relatively confined alpine valley with homogeneous climate and soil conditions. Additionally, the exploratory design of the study may be considered relatively small. However, it included three cultivars, four treatment replicates, and two growing seasons. The most important limitation of this study is the absence of must and vinification replicates—following harvest, the grapes from the treatment replicates were pooled together for a single vinification. The must sugars for C isotope analysis were separated from the pooled berries per treatment. To avoid this limitation, it would be necessary to produce must and wines per treatment block ($n = 4$), treatment ($n = 4$), variety ($n = 3$), and season ($n = 2$), resulting in 96 separations and purifications of must sugars and microvinifications. This considerable effort was beyond the scope of this exploratory study. These limitations (no field replicates for must and wines) were the same affecting the 2009–2014 irrigation experiments (Spangenberg et al., 2017; Spangenberg and Zufferey, 2018).

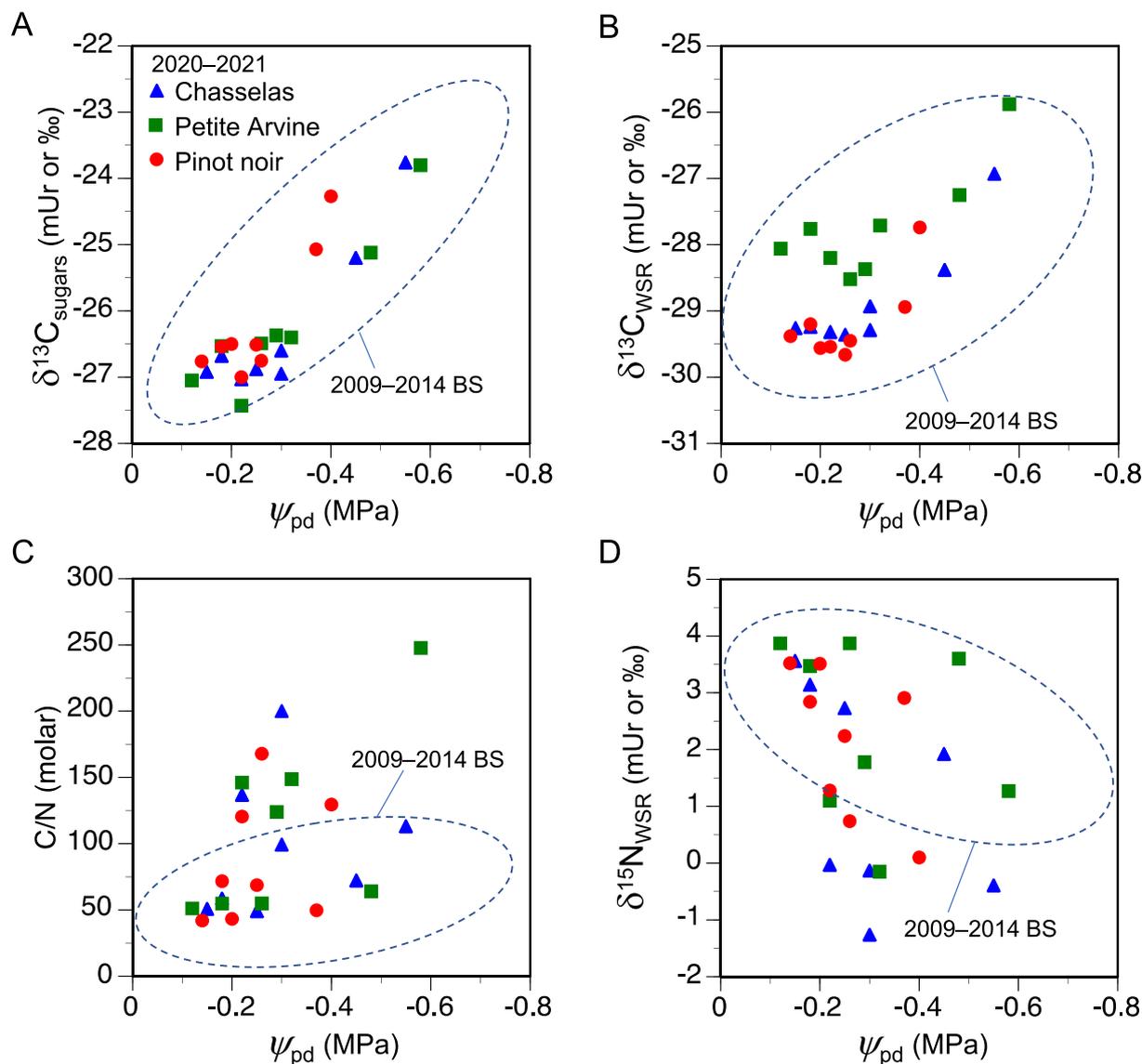


Fig. 9. Comparison of the $\delta^{13}\text{C}_{\text{sugars}}-\Psi_{pd}$ (A), $\delta^{13}\text{C}_{\text{WSR}}-\Psi_{pd}$ (B), $\text{C}/\text{N}-\Psi_{pd}$ (C), and $\delta^{15}\text{N}_{\text{WSR}}-\Psi_{pd}$ (D) relationships for Chasselas, Petite Arvine, and Pinot noir vines grown under different soil management practices (BS, GS) and water treatments (DI, NI) during 2020 and 2021. Ψ_{pd} is the abbreviation for predawn leaf water potential. Areas within dashed lines enclosed the values obtained for the same vines grown on bare soils at the same site under different soil water treatments during the 2009–2014 seasons (data from Spangenberg et al., 2017; Spangenberg and Zufferey, 2018, plotted in Fig. S4 to define the fields).

4. Conclusions

This study used an exploratory design to investigate the combined effects of cover cropping and water availability on the carbon and nitrogen levels and stable isotope composition of the WSRs from three grapevine cultivars grown under the same climate and soil conditions. The $\delta^{13}\text{C}_{\text{WSR}}$ and $\delta^{15}\text{N}_{\text{WSR}}$ values correlated well with the stem water potential and the $\delta^{13}\text{C}$ values of must sugars for the three studied grapevine varieties (Chasselas, Petite Arvine, and Pinot noir) over two consecutive seasons (2020–2021). The C/N molar ratios and the $\delta^{15}\text{N}_{\text{WSR}}$ values varied strongly with soil water availability and management, indicating an exacerbation of vine-weed competition for N in water-deficient vines. These molecular and isotopic values of wine residues showed a better resolution and separation of the vine groups from different soil management practices and irrigation treatments compared to the field-measured leaf chlorophyll index and the YAN in grape juices. The dual isotope approach not only serves to characterize the water-availability effects on wine but also the degree of N deficit in vines due to the combined effects of soil dryness and weed competition for water and nutrients. Pinot noir vines appear to adapt

much better than white grapevines to soil water and N deficits. Chasselas and Petite Arvine vines need to be well watered to avoid deficient uptake of soil N. Drip irrigation is not a sustainable solution, but as an agroecological solution, minimal and efficient irrigation (i.e., with rainwater, lake or river water) will significantly decrease the dose of applied herbicides. This action will alleviate the pressure on winegrowers regarding the time needed for the development of sustainable nonchemical soil management. A possible solution is the introduction of genetically modified grapevine cultivars with higher tolerance to pathogens and high N use efficiency.

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CRedit authorship contribution statement

Jorge E. Spangenberg: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Vivian Zufferey:** Conceptualization, Methodology, Writing – review & editing.

Data availability

All data from this study are provided within the manuscript and its Supplementary Material.

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the study reported in this paper.

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