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The potential of integrating biochar and stable isotope technologies for regenerative viticulture under climate change

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Abstract

Purpose Climate change is driving adaptation in vineyard management, with increasing focus on sustainability and long-term soil resilience. In Australia, the development and implementation of sustainable vineyard management practices are encouraged by the Australian Wine Industry, supported by national industry initiatives and certification programs. This review defines regenerative viticulture (RV), with a focus on soil microbe – plant – soil system dynamics and the role of biochar as a management intervention.

Materials and methods Within the RV framework, and with biochar application as the central management tool, this review synthesises the key biogeochemical mechanisms affecting vineyard soil health, particularly soil carbon (C) dynamics, nitrogen (N) transformations and soil water availability. It explores the prospective use of industry waste as a biochar source, and highlights the potential of stable C, N and oxygen (O) isotope compositions or natural abundance methods, alongside water extractable C and N pools, to fingerprint and quantify interactive soil C, N and hydrological processes.

Discussion This review highlights the potential of biochar application for developing a superfunctional platform to enhance soil biodiversity and ecosystems functioning (particularly C, N and hydrological cycles) for RV in Australia and elsewhere. The use of stable C, N and O isotope compositions and water extractable C and N are proposed as novel tools to monitor and advance a deeper understanding of the key soil-plant-microbe interactions within RV systems.

Conclusion This study highlights the potential of implementing biochar-based technologies as a key tool to promote soil biodiversity and ecosystem functions in the context of climate change, which can be fingerprinted by innovative isotope natural abundance methods for RV futures in Australia and elsewhere.

Keywords Regenerative viticulture · Biochar · Climate change adaptation · Australian viticulture · Stable isotope compositions · Functional microbes

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1 Introduction

Climate change has intensified extreme climatic events globally, including floods, droughts and heat waves (FAO 2020) with major implications for vineyards globally (Diffenbaugh et al. 2011; Webb et al. 2012; Hannah et al. 2013). Climate change, combined with conventional vineyard management, may compound to negatively impact vineyard soil health, microbial diversity and destabilise the vineyard ecosystem, putting vines under increased abiotic and biotic stress, jeopardising vine health and grape quality (O'Brien et al. 2025). In Australia, projected warming and increasing climate variability are expected to disrupt grapevine phenology, compress harvest windows, and change regional yield (Webb et al. 2012; Puga et al. 2023). This progression of declining vineyard functioning under climate

stresses underscores the need for management approaches that restore soil health and resilience, such as regenerative viticulture (RV).

The RV is emerging as a promising ecologically driven management approach to target restoration of soil carbon (C), nitrogen (N), and hydrological cycles alongside microbial biodiversity and reduction of agrochemicals and tillage to buffer vines against climate extremes. This approach simultaneously improves soil and vine health and resilience to support vineyard ecosystem services which can reduce pathogenic vulnerabilities and could potentially secure yield and berry quality, particularly in difficult years (Kesser et al. 2023; O'Brien et al. 2025). Although there are numerous short-term and long-term adaptive methods that vineyards may employ (Diffenbaugh et al. 2011; Webb et al. 2012; Reganold and Wachter 2016; Knapp and van der Heijden 2018; Rogiers et al. 2022), this review highlights the short- and long-term benefits that biochar offers.

Biochar is a C dense material formed by the pyrolysis of organic matter at high temperatures under limited oxygen or oxygen-free conditions (Joseph et al. 2010). It is highly recalcitrant in nature meaning that after application to certain soil types it can stay stable in the soil up to thousands of years making it a viable and long-term stable form of C storage that would otherwise be released to the atmosphere (Swift 2001; Lehmann 2007; Liang et al. 2008; DeLuca et al. 2012; Glaser et al. 2014). Biochar has an advantage over other C inputs like mulch and cover cropping in that it offers immediate benefits, without any known long-term toxic effects, for soil microbial communities and functioning, plant performance and crop production without the need for constant reapplication (Agegnehu et al. 2016; Maienza et al. 2017; Gross et al. 2024). However, over time, changes in the microbial communities can alter microbial activity to increase utilisation of recalcitrant C and use less of the more readily available carbohydrate C (Giagnoni et al. 2019). Yet the full scope of biochar impact has yet to be determined on the soil-microbe-vine interactions on different spatial and temporal scales, this is where the use of stable isotopes can be utilised.

Stable isotope natural abundance method is a powerful method that uses stable C, N and O isotope compositions for fingerprinting and quantifying the important and interactive processes of C, N and hydrological cycles to understand these biogeochemical processes in the soil-plant-climate systems (Prasolova et al. 2000; Huang et al. 2008; Wang et al. 2015; Succarie et al. 2022).

This review evaluates biochar's potential as a key RV tool for Australian vineyards under climate change and introduces stable isotope-based and biologically sensitive approaches for soil and vine monitoring.

1.1 Australian vineyards under climate change

Global warming is intensifying the hydrologic cycle, increasing the duration and intensity of droughts and occurrence of heat waves (Succarie et al. 2022). In the coming decades, Australia will experience increased temperature, heat extremes, drought, dangerous fire weather days and more intense short-duration heavy rainfall events (flash floods) which will have a profound effect on the lives and incomes of Australians ("State of the Climate 2024" 2024).

Emerging challenges facing Australian vineyards include heat stress, bushfires, water limitations and logging, heat-related berry stress, altered berry composition, increased pest and disease pressures, and soil degradation (Rogiers et al. 2022). As extreme climatic events extend in length and severity, there exists uncertainty of the full impact this will have on plant, soil, and soil microbial resilience for survival (Dhankher and Foyer 2018). By 2050, yield impacts are expected to vary regionally, with little change for area-weighted average yield across Australia, but higher yield in cooler regions and a lower yield in the hotter regions (Puga et al. 2023).

Industry-led initiatives such as *Sustainable Winegrowing Australia* and tools like the *Climate Atlas* underscore the sector's growing focus on sustainability and resilience (Harris et al. 2020; "Australian Wine Sector at a Glance" 2023). There is growing interest in Australian vineyards to adapt their practices to future climate change scenarios and in the usefulness of climate change analogues for long term decision making (Dunn et al. 2015). This aligns with increasing consumer willingness to pay for biodynamic, fairtrade, organic, natural or sustainable wines (Gow et al. 2022). Therefore, there exists a need for adaptive vineyard management to rebuild soil health and ecosystem functioning to buffer vines against increasing abiotic and biotic stresses and meet consumer expectations. The RV provides a soil-centric framework to rebuild vineyard resilience and meet these climate and market challenges.

1.1.1 Vineyard soil health

Soil health is defined as the capacity of soil to function as a living system that sustains productivity, environmental quality, and ecosystem services through physical, chemical and biological indicators (Doran and Zeiss 2000). It integrates physical, chemical and biological indicators including soil structure, nutrient cycling and availability, soil microbial diversity and activity, and macro and mesofauna (Das and Varma 2010; Cardoso et al. 2013). These characteristics are largely reflective of the geographic circumstances and "inherited qualities" of that soil (Das and Varma 2010). In Australian vineyards, soil health underpins

vine performance, disease resilience, yield and berry quality (Rogiers et al. 2022).

Australian vineyards typically operate on ancient and nutrient-poor soils (Oliver et al. 2013), with their 'inherited qualities' strongly influencing vine growth and berry composition. For example, soil cation chemistry significantly affects berry juice Baumé and titratable acidity of grape juice as demonstrated by a study of the mineralogy of two Riesling vineyards in South Australia (Mackenzie and Christy 2005). In healthy agroecosystems, soils preserve environmental integrity by stabilising nutrient supply and soil structure, promote microbial communities and functioning, resist pathogens, parasites and weeds and reduce degradation (Magdoff 2001; He et al. 2009; Xu et al. 2009). Recent field studies in South Australia demonstrate that under-vine cover crops can increase soil organic C by over 20% and more than doubled microbial activity over five years (Marks et al. 2022), highlighting the importance of soil-focused management and monitoring in Australian viticulture.

Australian vineyard soil could be measured and monitored using key factors: aggregate stability, air-dry soil consistence, pH, electric conductivity (EC), cation exchange capacity (CEC), exchangeable cations (ExCs) and total organic C (TOC) to develop a national vineyard soil quality monitoring system minimum data set (Oliver et al. 2013). In addition, stable isotope natural abundance alongside labile fractions of soil C and N, such as water-extractable organic C (WEOC) and water-extractable total N (WETN), provide sensitive key indicators of land management and soil biogeochemical processes revealing microbial activity, disturbances, short-term nutrient availability, and soil functional recovery (Bai et al. 2015; Wang et al. 2020; Succarie et al. 2022; Yang et al. 2023; Kichamu-Wachira et al. 2024). Overall, it is difficult to apply a national vineyard soil quality monitoring system consistently due to regional variation and lack of standardised indicators. For example, in a meta-analysis of cover cropping and tillage, only 8 of 42 identified soil Health indictors were reported more than 20% of the time (Stewart et al. 2018). However, developing an vineyard specific benchmark for different regions like the "Soil Health Gap" approach comparing nearby undisturbed soil with managed soils (Maharjan et al. 2020), alongside improved soil organic carbon (SOC) monitoring, reporting and verification platforms (Smith et al. 2020), offer pathways to improve vineyard soil health monitoring in Australia.

1.1.2 Role of C in vineyard soil

Soil organic C (SOC) is a critical driver of soil health, positively correlated to improved soil structure and ecosystem services, and increased soil microbial activity, nutrient cycling and water holding capacity (WHC) (Lal et al. 2007; Lehman et al. 2015). High SOC improves vineyard soil stability and resilience, supporting vine growth and buffering against climatic extremes (Visconti et al. 2024), while depleted SOC is a hallmark of degraded soils with reduced biological function and high input demands (Lal 2012). Higher SOC also improves microbial C use efficiency (CUE), the ratio of microbial growth over C uptake, while promoting the conversion of organic substrates into stable soil C pools while sustaining nutrient cycling (Manzoni et al. 2012; Wang et al. 2021; Tao et al. 2023). As organic matter decomposes, it releases labile C forms that serve as nutrient sources and energy or substrate for soil microbes (Landgraf et al. 2006; Garcia-Pausas and Paterson 2011). Stable C isotope ($\delta^{13}C$) analysis provides a diagnostic tool for assessing CUE and soil organic matter dynamics, linking C inputs to soil microbial processing efficiency (Werth and Kuzyakov 2010).

Grape vines are largely grown in regions with notoriously poor and degraded soils which would benefit from the addition of C to the soil through the implementation of cover crops, mulch, manure, compost and biochar application (Guerra and Steenwerth 2012; Priori et al. 2018; Visconti et al. 2024; O'Brien et al. 2025). Vineyard studies have found that cover crops (Peregrina et al. 2010; García-Díaz et al. 2018) and organic applications (manure and green manure) (Morelli et al. 2022) increase soil labile C, instantly increasing soil fertility. A six-year field trial in South Australia found that under-vine cover-cropping increased leaf area index (LAI) and fruit yeast assimilable N (YAN) without reducing yield, compared to herbicide and native perennial Wallaby Grass treatments (Lines et al. 2024). However, for optimal vineyard results, the reduction of pests and disease, and to control potential water competition, the selection of cover crop species and application of other organic materials, requires careful consideration of numerous factors including regional climate, soil type, onsite disease and pests and nearby land management practices (Danne et al. 2010; Lines et al. 2024). Biochar has one advantage here over other C inputs like mulch and cover cropping, it offers immediate benefits for plant performance and crop production without the need for constant application (Agegnehu et al. 2016; Gross et al. 2024). It also has no known long-term toxic effects on existing soil microbial communities have been found (Maienza et al. 2017). However, over time, changes in the microbial communities can alter microbial activity to increase utilisation of recalcitrant C and use less of the more readily available carbohydrate C (Giagnoni et al. 2019).

The C sequestration also contributes to global climate mitigation goals. Increasing SOC removes CO_2 from the

atmosphere and locks it in stable soil pools, improving soil health while aligning with sustainability initiatives (Lal 2004, 2008; FAO 2019). Biochar, a highly recalcitrant C source, is particularly promising for long-term C storage in soils (Joseph et al. 2010; Woolf et al. 2010) whilst delivering both short- and long-term benefits aligning with RV principles (Baronti et al. 2022; Kingston et al. 2024; O'Brien et al. 2025).

1.1.3 Role of N in vineyard soil

Although N is abundant in the atmosphere, it must be biologically or chemically, via fertiliser applications, transformed into plant-available forms via microbial processes including biological N fixation (BNF), N mineralisation, nitrification and denitrification before utilisation (Robertson and Groffman 2007; Stein and Klotz 2016; Reverchon et al. 2020). As a key component of plant amino and nucleic acids, and chlorophyll, N is the third most abundant element in plants after C and oxygen (O), making it a key limiting nutrient in terrestrial and agricultural systems (McNeill and Unkovich 2007; Sun et al. 2010, 2024; Hosseini Bai et al. 2012; Reverchon et al. 2020; Li et al. 2024). Is it an essential macronutrient for grapevine growth and fruit composition, influencing vine vigour, canopy density, berry size and yeast assimilable N (YAN) (Bell and Henschke 2005).

Vineyard soils are often low in available N and require careful management to balance vine growth with berry quality. Effective N management improves N use efficiency (NUE), ensuring greater alignment between plant N demand and soil N supply while reducing environmental losses (Fageria and Baligar 2005; Govindasamy et al. 2023). Measurements of labile N factions are useful sensitive indicators of soil biological activity and short-term N cycling (Wang et al. 2020; Yang et al. 2023). The ^{15}N natural abundance ($\delta^{15}N$) is another powerful tool to trace N transformations and NUE, identifying N transformation pathways and loss in vineyard soils which can inform management practices (Santesteban et al. 2024).

Soil microbial communities underpin these N cycling transformation processes. Soil bacteria, archaea, and fungi drive all major stages of N cycling and are responsible for the quantity, speciation and bioavailability of N (Cayuela et al. 2013; Offre et al. 2013; Lehman et al. 2015; Ning et al. 2015; Zhang et al. 2017, 2018a; Che et al. 2018). Nitrification is undertaken by autotrophic nitrifying microorganisms including both ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB), and heterotrophic nitrifiers (Amoo and Babalola 2017). Denitrification is undertaken by denitrifiers and is a functional trait found across diverse bacteria, archaebacteria and fungi taxa (Zumft 1999; Bloem et al. 2005; Ambus and Zechmeister-Boltenstern 2007). The four known main fungi, arbuscular mycorrhizal (AM), ectomycorrhiza (EcM), ericoid mycorrhizal (ErM) and orchid mycorrhiza (OrM) are mostly involved in heterotrophic nitrification and denitrification providing N to approximately 97% of known terrestrial plants through symbiosis (Brundrett and Tedersoo 2018). The AM fungi (AMF) obtain recalcitrant and labile forms of organic N from SOC, as well as inorganic N from the soil and deliver N to host plants in exchange for C (Whiteside et al. 2012). EcM can oxidize SOM to access the organic N through several different decomposition methods (Nicolás et al. 2019).

The N-functional genes for nitrification (*amoA*) are found in AOB and AOA, and denitrification genes (*napA*, *narG*, *nirK*, *nirS*, *nosZ*) are useful to link soil microbial N cycling to N_2O emissions (Levy-Booth et al. 2014). Due to these genes sensitivity to climate change, N_2O emissions can increase to 33% under hotter temperatures, and 55% under increased precipitation, while inhibited under drier conditions (Li et al. 2020). Microbes have high surface to volume ratio, respond quickly to environmental changes making them excellent indicators of changes in soil health (He et al. 2009; Cardoso et al. 2013; Che et al. 2016).

The macro- and meso-pores within the biochar structure provide an excellent environment for soil microbial communities (Lehmann et al. 2011) and increase soil bacterial diversity and community structure (Chen et al. 2013; Xu et al. 2016). However, it may also reduce or retain pre-biochar amounts of soil microbial activity and abundance (Ameloot et al. 2014). Biogeochemical N transformations can be affected by both biotic (microbial activities) and abiotic (chemical e.g., sorption) processes (Dai et al. 2020). As soil microbial populations increase with biochar application, N_2O fluxes may increase due to a stimulation of denitrification, or a decrease due to an increase in the production and sorption of ammonium (NH_4^+) onto the biochar surface (Anderson et al. 2011). It has been hypothesised that biochar can act as a 'N island' that regulates N cycling fluxes by reducing N loss (Anderson et al. 2011; Li et al. 2024; Sun et al. 2024). However, soils may have a dominating N_2O pathway which determines whether biochar application will increase denitrification by decreasing the $N_2O/(N_2+N_2O)$ ratio or increase nitrification and N_2O emissions (Sanchez-Garcia et al. 2014). Although a small amount of biochar can increase biological N fixation (BNF), higher amounts of biochar may suppress N_2 fixation (Horel et al. 2018a). As a climate change mitigation strategy, biochar has the potential to reduce global anthropogenic greenhouse gas emissions (e.g. N_2O) by reducing the need for fertilisation whilst improving soil fertility and primary production (Joseph et al. 2010; Zhang et al. 2010; Brunetto et al. 2016).

The complex N interactions between soil – soil microbe – vine – berry mean that optimal N fertilisation in the

vineyard would vary depending on changing requirements during the different phenological stages to address vigour, grape composition, limit pollution and control production costs (Verdenal et al. 2021). Ideally this would be guided by analysis of petiole N, vine vigour, canopy density and leaf colour (Bell and Henschke 2005). By targeted N fertilisation, soil N retention would increase and reduce potential emission of the potent greenhouse gas N_2O (Longbottom and Petrie 2015). The implementing of C rich soil amendments like biochar, compost, mulch or other organic amendments can effectively increase total N in degraded vineyard topsoil (Horel et al. 2018a; Priori et al. 2018; Kingston et al. 2024).

The tight coupling between soil N and C dynamics and increasing vine demand for N under climate change entails that N management in RV should integrate soil health centric practices like biochar application to stimulate soil microbial-mediated N cycling.

1.1.4 Role of water in vineyard soil

Reduced precipitation and higher temperatures will intensify evaporation, Making water increasingly limiting in Australian vineyards. This will require greater irrigation use, placing pressure on water resources in an already dry country that may impact on the environmental water balance and hydrological cycle. Future climate modelling in South Australian vineyards predicts irrigation requirements may rise by 3.5–7.5% before veraison to avoid impaired canopy development during flowering and berry development (Phogat et al. 2020). Under climate change, reduced precipitation in Australian vineyards will necessitate increased irrigation in regions like the South Australian Riverlands, risking rootzone salinity affecting growth, yield and wine quality (Phogat et al. 2018) and potentially increase downstream N run-off (Barlow et al. 2009). Strategies like regulated deficit irrigation (RDI) and partial root zone drying irrigation (PRI) can increase water use efficiency (WUE) and improve berry quality in Mediterranean-type vineyards (Romero et al. 2022). This may suit the dry summer climate of Australia's southern wine grape growing regions but may not be as feasible for Queensland's wetter summer climate.

The physiochemical properties of biochar can impact vineyard soil by increasing water holding capacity (WHC) of specific soils (Anderson et al. 2011; Case et al. 2012; Xu et al. 2016). This is relative to the biochar's macro- and meso-pore size which are dependent on the raw material used (Marshall et al. 2019). Biochar WHC is also impacted by pyrolysis temperature, with aliphatic and aromatic compounds removed at higher temperatures, decreasing its hydrophobicity (Marshall et al. 2019) and increasing its WHC (Anderson et al. 2011; Case et al. 2012; Xu et al. 2016). Biochar retention of plant accessible water is important for vines undergoing water stress (Baronti et al. 2014), with application to crops in drought prone areas showing positive outcomes for crop productivity, particularly in years of low rainfall (Genesio et al. 2015). Biochar also increases water filled pore space (WFPS). While WHC measures the soils capacity to retain water after drainage, WFPS refers to the dynamic changes in water within pore space at any one time subject to WHC, soil texture and moisture present. Increased WFPS is linked to improved crop yield, however, depending on soil type, also increases soil N_2O emission (Deng et al. 2015).

Other agronomic strategies that improve soil moisture retention through mulching, cover-cropping, canopy management, and targeted irrigation are essential, as is thoughtful cultivator and rootstock choice, are essential for improving plant WUE in grapevine production (Medrano et al. 2015; Marín et al. 2021). The adoption of more water efficient rootstock and scions alongside good soil and canopy practices is a suitable and practical option for growing regions which do not have adequate rainfall or irrigation sources (Medrano et al. 2015). Borghi et al. (2024) propose selecting and adapting endophytes from drought-resilient vines for inoculation in other regions as a sustainable strategy to improve WUE. Unlike irrigation-based approaches, this method enhances vine drought tolerance biologically, promoting root growth and water uptake without increasing external water demand. Stable O and hydrogen (H) isotopes ($\delta^{18}O$ and $\delta^{2}H$) provide complementary tools to assess plant WUE and soil-plant water relations, enabling monitoring of irrigation efficiency and drought response (Ehleringer and Dawson 1992; Cernusak et al. 2016). These isotope compositions are also applied to identify plant water sources and verify wine provenance and authenticity, a valuable tool for viticulture (Camin et al. 2015).

Addressing the interconnection among soil C, N and hydrological cycles in the vineyard in a sustainable manner requires management shifts towards rebuilding of soil health and functioning, and ecosystem resilience. The integration of biochar with other organic C inputs like cover cropping and mulch offer a RV approach grounded in soil health and ecological principles to support climate adaptation in vineyard systems (Fig. 1).

1.2 Regenerative viticulture

The RV burst onto the international scene is the late 2010's. It is a soil-centric vineyard management approach designed to build soil C, restore ecosystem functioning, boost vine health and resilience, and enhance biodiversity while maintaining a profitable grape production (O'Brien et al. 2025). Originating from regenerative agriculture (RA) principles

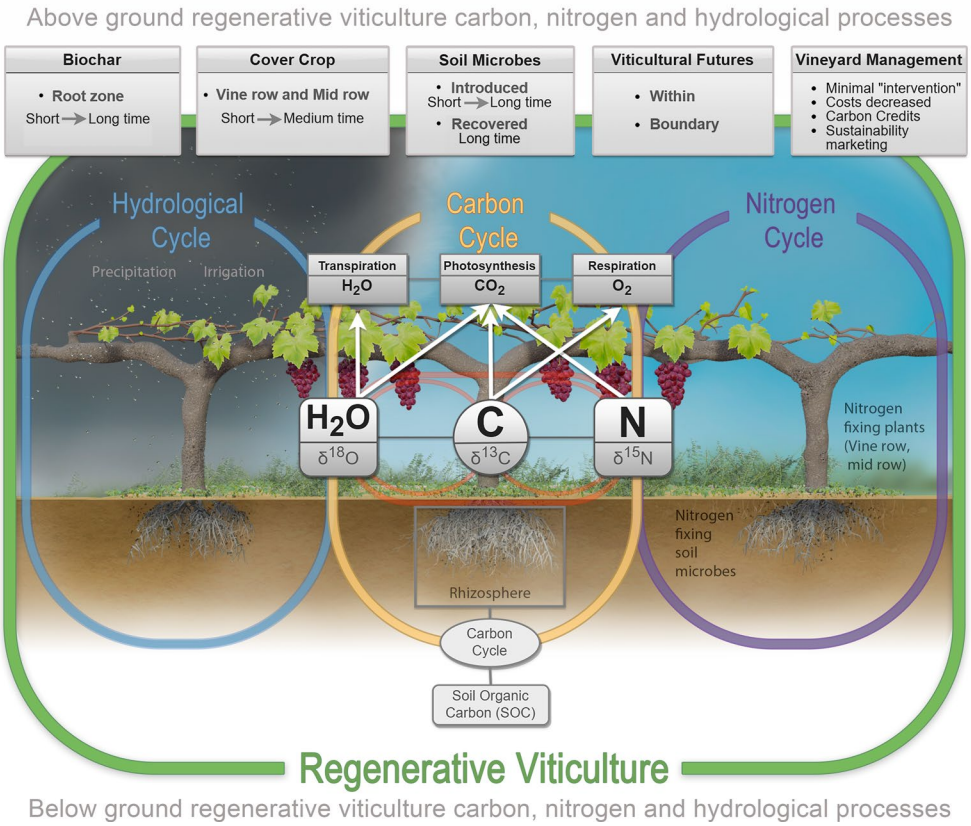

Fig. 1 Conceptual framework illustrating the key components of regenerative viticulture (RV), highlighting the integration of biochar, native microbial inputs, cover cropping, and soil–plant–climate interactions across the hydrological, carbon and nitrogen cycles. Stable isotope tracers ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) represent tools to monitor these linked processes within the vineyard system

(Rodale 1983; Newton et al. 2020), RV prioritises soil health as the foundation for vine resilience, climate adaptation, and long-term sustainability (Visconti et al. 2024). Defining RV requires the consideration of the core concepts of RA and their transferability to the vineyard. The RV is a multi-dimensional environmental restoration approach to regenerating vineyard soils to optimum health and functioning and increase ecosystem services, primarily through increasing soil C and biodiversity.

Conventional viticulture, like conventional agriculture, is characterised by intensive tillage, synthetic fertilisers, agrochemicals, and simplified monocrops which impact soil physio-chemical properties (Ferreira et al. 2020; Visconti et al. 2024). In contrast, RV employs ecologically based practices, like that of conservation, sustainable and organic agriculture, to reduce soil disturbance, improve nutrient systems, biodiversity and strengthen ecosystem services (Pittelkow et al. 2015; Reganold and Wachter 2016; Knapp and van der Heijden 2018; O'Brien et al. 2025).

The RV integrates RA principles such as reduced tillage, permanent ground cover, organic amendments (e.g. cover crops, mulch, biochar), integrated pest and nutrient management, and biodiversity (LaCanne and Lundgren 2018; Lal 2020; Khangura et al. 2023; O'Brien et al. 2025). The enhancement of soil C until saturation to stabilise soil C for long-term storage and minimal leakage is an important soil regeneration principle (Lal 2020). However, constantly disturbed soils may never reach absolute C saturation (Stewart et al. 2007), but lower vineyard management (no tillage or herbicide) can improve soil health and vineyard ecosystem functioning (Kesser et al. 2023). The RV principles can rebuild soil structure, enhance soil microbial diversity, improve nutrient and water retention and buffer vines against climate extremes (O'Brien et al. 2025). Early adoption in Australian vineyards of RV soil-centric, ecological management principles is supported by Wine Australia's 'EcoVineyards' program and the Australian Wine Research Institute (AWRI).

Currently, there are few studies that focus on RV. Recent reviews, including O'Brien et al. (2025), highlight RV's potential as a climate change adaptation pathway for vineyards. While long-term empirical data remain limited due to its novelty, the core practices of cover cropping, organic amendments and biodiversity integration, are strongly linked to improved water retention (Medrano et al. 2015) and increased soil C stocks and biodiversity (Woolf et al. 2010; Giffard et al. 2022; Pilar et al. 2022). These mechanisms directly address vineyard issues in relation to projected warming and drying trends for Australian vineyards.

Although evidence specific to RV under future climate scenarios is still emerging, its soil focused strategies align with vineyard adaptive requirements. By enhancing soil C,

improving WHC, increasing biodiversity, and supporting soil microbial processes, RV offers an informed framework to mitigate climate risk and build resilience in vineyards (O'Brien et al. 2025). By increasing C pools and C sequestration using stable long lasting C dense materials like biochar (Lal 2020), have large scale climate change mitigation potential (Woolf et al. 2010). By aligning biochar requirements to local soil types and climates, strategic use of region appropriate cover-crop species (Lines et al. 2024) could increase SOC, suppress weeds (Kunz et al. 2016), and boost biodiversity (Vogelweith and Thiéry 2017).

1.3 Potential of biochar in the vineyard

Biochar is increasingly recognised as a stable long-term C input that enhances soil health without compromising key grape quality parameters (Baronti et al. 2014; Genesio et al. 2015). With application, it can alter soil physical properties, including increasing total C (TC) and the C: N ratio (Case et al. 2012; Nessa et al. 2021; Sun et al. 2024), reducing soil bulk density (Laird et al. 2010; Zhang et al. 2010; Genesio et al. 2015), increase soil aeration (Anderson et al. 2011). It can increase soil pH due to its alkalinity and negatively charged surface area (Zhang et al. 2010; Case et al. 2012; Giagnoni et al. 2019) and slowly release fertiliser nutrients for plant uptake (DeLuca et al. 2012; Viger et al. 2015; Brunetto et al. 2016).

Despite its benefits, biochar may have some limitations. Kavitha et al. (2018) consolidated several potential drawbacks of biochar application. Its effects can be highly soil-specific, may increase weed proliferation, and aged biochar has been linked to negative impacts on earthworm and fungal growth. Additionally, biochar can inhibit soil aging, disrupt optimal nutrient cycling, reduce root biomass, interfere with SOM decomposition, and lower soil thermal diffusivity. Kavitha et al. (2018) also highlight that biochar's impact on plants varies with application rates, as different species respond differently in terms of growth and flowering. Plant development can further be influenced by the selective absorption of pollutants, high pyrolysis temperatures that alter biochar properties, and interactions that either sequester or react with essential nutrients. In some cases, biochar may introduce contaminants that negatively affect plant growth. As a form of recalcitrant C, it cannot be used as a form of fertilizer but only function as a reservoir for the slow release of nutrients (Kookana et al. 2011). Practical considerations exist in terms of accessibility and affordability. More research is needed in terms of how biochar will impact soil, microbes and plant function under extreme climatic conditions that will become commonplace in the future and to address any limitations as a RV method.

1.3.1 Biochar in vineyard soil management

Field-based studies of vineyard soils highlight biochar's capacity to restore soil function and its impact on vine and berry. Although there have been a number of recent international studies in Europe (Genesio et al. 2015; Maienza et al. 2017; Baronti et al. 2022; Idbella et al. 2024) and North America (Verhoeven and Six 2014; Garcia-Jaramillo et al. 2021) evaluating biochar as a viable soil amendment in vineyards, there are comparatively few studies undertaken in Australian vineyards to date. In a multi-year vineyard trial in Italy, biochar increased SOC and WUE, and improved vine physiology performance including higher leaf water potential, stomatal conductance, and photosynthetic rate (Baronti et al. 2014; Schmidt et al. 2014). Similar results are reflected in a potted grape vine study that highlighted biochar impact on increasing field capacity and plant available water (Petrillo et al. 2020). Incubation experiments on Australian vineyard soil showed biochar increased WHC, SOC, N retention and reduced N losses (Kingston et al. 2024).

Biochar has the potential to address some fungal and parasitic issues that arise in vineyard soil. A two-year field trial that applied poultry litter biochar to vineyard soil in New South Wales showed a decrease in the total plant-parasite nematode population while increasing the total non-plant parasitic nematode populations (Rahman et al. 2014). It also demonstrated beneficial impacts on vineyard soil microbial communities with more effective results when applied as a biochar-compost combination reporting an increase in soil microbial abundance and functional activity for up to 20 months following application (Mackie et al. 2015).

In addition to improving soil health and vine performance, biochar application may also reduce greenhouse gas emissions from vineyards. Traditional agricultural practices like tillage were commonly used in vineyards last century to reduce weeds which increased soil CO_2 and N_2O emissions, however applying biochar can decrease N_2O emissions with some small changes in CO_2 release (Horel et al. 2018b). It is important to note that under some circumstances and depending on soil properties, biochar application may increase N_2O emissions at vineyard scale (Verhoeven and Six 2014).

1.3.2 Vineyard waste as a biochar source

Grape marc and vine prunings are two of the largest by-products of viticulture, offering a potentially valuable, sustainable source of biochar feedstock. Grape marc is the residue left after juice extraction, it contains seeds, skins,

pulp, and stems and is often used for ethanol distillation, producing exhausted grape marc (EGM). Without sustainable management, EGM is an environmental hazard contributing to nutrient leaching, surface and groundwater pollution and emitting foul odours as it degrades (Xu et al. 2009; Jones et al. 2020).

Recent studies have explored the pyrolysis of EGM and vineyard prunings to produce stable, nutrient-rich biochars suitable for soil amendment. Frikha et al. (2021) found that pyrolysis temperatures of EGM between 450 °C and 550 °C produce an agronomically beneficial, nutrient-rich biochar, of which the 450 °C EGM biochar showed the most benefit for ryegrass growth. Similarly, Ferjani et al. (2020) reported that at increased pyrolysis temperature (500 °C) of EGM biochar, phosphorus (P) is stabilised via possible complexion, however, potassium (K), sodium (Na) and calcium (Ca) are converted into more leachable and plant available forms, enhancing its potential as a slow-release fertiliser.

Pyrolysis temperature of grapevine cane and stalks strongly influences the physical properties of vineyard-derived biochar. Depending on the feedstock and pyrolysis conditions, biochar yield from grapevine wood ranges between 25% and 35% (Rosas et al. 2015), making it an attractive method for sustainably repurposing old vines. Marshall et al. (2019) observed that biochar produced from grapevine cane and stalks at lower temperatures (400 °C) had higher hydrophobicity, while those produced at higher temperatures (700 °C) exhibited optimal available water content (AWC) and therefore, greater potential for water retention. Furthermore, high-temperature grapevine cane at high pyrolysis temperatures may improve WHC of coarse-textured, sandy vineyard soils (Marshall et al. 2019), which could be particularly beneficial for the soils of the Granite Belt region in Queensland of subtropical Queensland.

1.3.3 Integration of biochar into RV

Biochar is most effective when incorporated into holistic, sustainable vineyard soil strategies. Combining biochar, cover cropping with the introduced functional microbes is a novel and innovative RV method. The addition of microbes from native pristine soils alongside biochar and cover cropping may stimulate soil N cycling, reduce N_2O and N loss as reported by Hiis et al. (2024). This complete integration could potentially address soil microbial community imbalances and disturbed functioning due to long-term agrochemical spraying and reduce the need for synthetic fertilisers. Due to the different responses of soil bacterial and fungal communities, unique to a vineyards biogeographic factors and management style (Likar et al. 2017), we propose that these microbes be sourced from local undisturbed native forest soils. From undisturbed habitats, these microbial communities are more likely to have functioning N cycling genes, high community diversity and abundance, and be well-adapted and resilient to the local climate improving the odds for a successful transferral. This process of introducing microorganisms into another environment, natural or man-made, to manipulate the rate of process is called bio-augmentation. Although it is often used in waste water treatment and remediation of heavy metals in soil, survival for microorganisms in these new environments is a challenge. He and Löffler (2024) produced a N_2O consuming bacterium, *Cloacibacterium* sp. strain CB-01 from enriching naturally occurring microbes by rotating them between agricultural soil, and partially treated sewerage, which when applied to farm soil reduced N_2O emissions from 50% to 95% depending on soil type and temperature (Hiis et al. 2024). We propose that by transferring community rich and abundant native soil microbes to vineyard soil amended with biochar providing suitable habitat, with water and nutrient retention, alongside cover cropping providing the native microbes with readily available form of C could be a future RV method of regenerating vineyard soil microbial diversity and functioning.

1.4 Potential of stable C, N and O isotope compositions for fingerprinting C, N and hydrological cycles in vineyards

The use of stable C, N and O isotope compositions offers a powerful approach to explore the soil-plant-microbe-climate interactions in C, N and H_2O cycles under different vineyard management approaches. Isotopic compositions of C ($\delta^{13}C$), N ($\delta^{15}N$) and oxygen and hydrogen ($\delta^{18}O$, $\delta^{2}H$) are measured using isotope ratio mass spectrometry (IRMS) expressed as parts per thousand (‰), and isotopic abundances reported as a ratio (δ) in relation to the appropriate international reference for that element. These techniques have been applied to characterisation of terroir and tracing geographical origins of wines (Di Paola-Naranjo et al. 2011; Dutra et al. 2013; Camin et al. 2015; Lancellotti et al. 2021; Saar de Almeida et al. 2023), and assess soil moisture variability, plant water status and impact of vineyard soil properties on wine (Brillante et al. 2018; Spangenberg and Zufferey 2018) (Fig. 2).

The use of $\delta^{13}C$ is well established as a measure of vine water status and WUE which can be measured through the $\delta^{13}C$ of grape sugars at maturity, and be a potential quality indicator (Gaudillère et al. 2002; Van Leeuwen et al. 2009; Coulouma et al. 2020). $\delta^{13}C$ reflects vineyard terroir differences (Santesteban et al. 2014), tracks vineyard soil erosion and degradation (Agata et al. 2015) and soil C dynamics (Balesdent et al. 1987), microbial CUE (He et al. 2024) and land use legacy (Dow et al. 2024). In regenerative vineyard

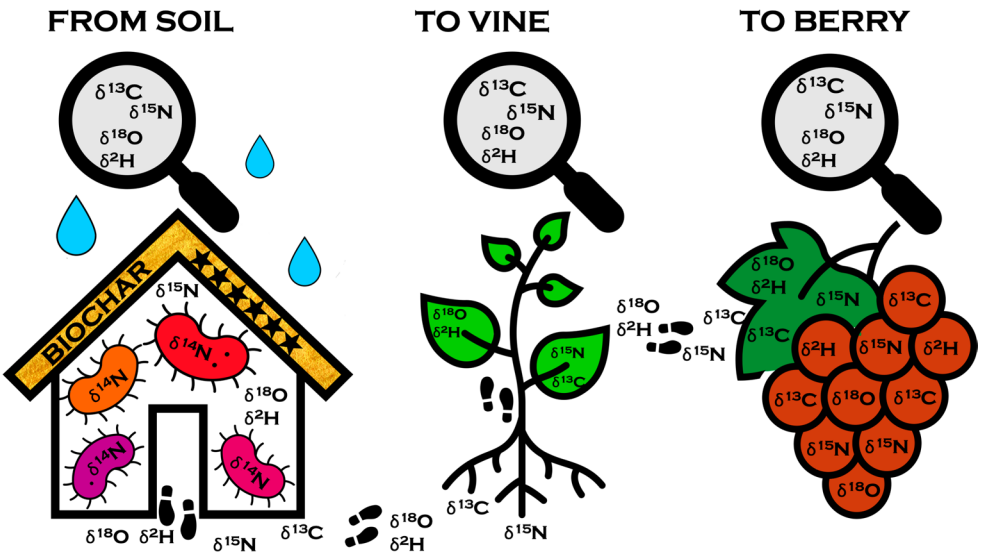

Fig. 2 A conceptual diagram illustrating the use of stable isotope compositions ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$) to highlight how the effects of biochar can be traced through the soil–vine–berry continuum within a RV system

soils, $\delta^{13}C$ offers a dual role: monitoring vine water stress and evaluating soil C turnover under biochar and other organic amendments like cover cropping.

The $\delta^{15}N$ signatures reflect N sources, microbial N transformations, and NUE. Although research is currently limited, the use of $\delta^{15}N$ may have potential to be an important berry quality indicator (Santesteban et al. 2024). In N cycling, when soil microbes are not limited by C, N and water, they discriminate ^{15}N in favour of the lighter ^{14}N as N is lost from the soil plant ecosystems leaving the soil enriched in ^{15}N (Wang et al. 2015; Zhang et al. 2018b; Choi et al. 2020; Succarie et al. 2022). This heavier ^{15}N is taken up by the vine and distributed across the varying sampling tissues and represents soil microbial N functioning and vine NUE. The $\delta^{15}N$ values were recorded as higher in vines subjected to organic fertilisers over inorganic fertilisers, and $\delta^{15}N$ values were reflective of differences in terroir, vineyard management and water availability (Santesteban et al. 2014, 2024). However, if microbes are under stress, they will utilise the heavier ^{15}N particularly under fungi dominated vineyard soils, leaving the soil with a negative ^{15}N value. Laboratory incubation experiment of Australian vineyard soils, show biochar application increased soil N cycling, N retention and reduced N loss, as evidenced by shifts in $\delta^{15}N$ of ammonium-N (NH_4^+-N), nitrate-N (NO_3^--N) and mineral N (NH_4^+-N+NO_3^-N) (Kingston et al. 2024). This study also recorded persistently negative $\delta^{15}N$ of NO_3^-N values suggesting potential legacy effects of fungicide use, altering microbial community and adaptation towards fungi-dominated communities that preferentially utilise ^{15}N.

Qian et al. (2025) demonstrated how historic N fertilisation has increased global N_2O emissions due to increased soil N availability and greater ratio of *nirS* abundance to *nosZ* abundance. This has significant implications for soil N cycling, as it may reduce the capacity of N cycling microbes to reduce N_2O, particularly in intensively managed agricultural systems like vineyards. Such findings highlight the importance of assessing N functional genes in evaluating the long-term sustainability of vineyard management strategies.

Both $\delta^{18}O$ and $\delta^{2}H$ can be used to trace vine water uptake pathways and link soil-water-climate interactions with grape and wine signatures. The $\delta^{18}O$ in vine tissues and berries can be traced from soil water, with enrichment related transpiration rate and changes traced to climatic events (Santesteban et al. 2015). As water has a unique isotopic signature reflected of its origins, $\delta^{18}O$ and $\delta^{2}H$ can be used for authentication of a wines geological origin (Gao et al. 2025).

The following novel and innovative approach are proposed as a method to trace the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$, $\delta^{2}H$ through the soil, leaf, petiole, berry and wine to determine the impact of RV methods on soil microbial activity throughout the entire soil-vine-wine cycle to develop a deeper in-depth understanding of the biogeochemical processes at play in the vineyard. Integrated RV applications of biochar, cover cropping and introduced microbes could potentially increase soil C and N cycling, nutrient and water retention for microbe and vine availability and reduce C, N and water losses. This can be identified by changes in the isotopic ratios of C and N (Fig. 1). Further research is recommended for soil DNA extraction to undertake real-time quantitative PCR to identify N functional genes in vineyard soils. Alongside a holistic ^{13}C, ^{15}N and ^{18}O analysis of soil and vine components and soil WEOC and WETN under this comprehensive RV management approach. Combining these three methods would provide a all-inclusive RV monitoring package to quantify the impact of biochar on the soil microbe – plant – soil system.

2 Conclusion

This review highlights the potential of biochar as a RV tool for enhancing vineyard soil health in Australia and beyond. Integrating biochar, especially in conjunction with cover cropping and introduced soil microbial diversity, has the potential to be a superfunctional platform for improving vineyard soil and ecosystem functions.

The use of stable isotope signatures of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ offers a powerful framework to fingerprint and quantify the interactive C, N and hydrological cycles within the soil – plant – microbe system in the vineyard. This, combined with the use of WEOC and WETN, presents additional promising tools for evaluating vineyard soil responses to biochar application in RV. The sensitivity of stable isotope signatures and soil labile analysis in response to C, N and water dynamics, seasonal shifts, moisture variation, and microbial turnover, makes them particularly valuable for assessing the biogeochemical impacts of these interventions within RV systems.

These findings collectively establish a strong foundation for future research. Building on this design, future studies could explore the incorporation of functional microbial communities sourced from native ecosystems to enhance nutrient cycling, microbial resilience, and berry quality under changing climate conditions.

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Declarations

Competing Interests Prof. Zhihong Xu, a co-author of this manuscript, is an Editor in the Journal of Soils and Sediments. Prof. Xu has requested that the handling and editorial responsibility for this manuscript be assigned to an alternative Editor. The authors declare that they have no other financial or non-financial competing interests.

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