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# Understanding carbon sequestration, allocation, and ecosystem storage in a grassed vineyard

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## Abstract

Understanding if and to what extent agricultural land types can sequester carbon is important for assessing their greenhouse gas mitigation potential. Grassed vineyards have recently been described as large carbon sinks relative to most cropland types, indicating the importance of understanding their carbon cycle in more detail.

To this end, we conducted a detailed study along a growing season in a grassed mountain vineyard with two varieties (Chardonnay and Sauvignon blanc) to quantify the overall carbon stock of the system and to attribute the carbon fluxes to the specific components of the carbon cycle of the agroecosystem, including vines organs (shoots, fruits, roots), grasses (shoots and roots) and soil. We combined eddy covariance, soil respiration, biometric measurements and soil analysis.

Our findings determined the studied vineyard to be a moderate carbon sink. We found a gross primary production ( $2409 \pm 35 \text{ g C m}^{-2}$ ) much larger than previous data for vineyards, but the net ecosystem production ( $246 \pm 54 \text{ g C m}^{-2}$ ) of the growing season was on the lower end of previous reports. The grassed alleys comprised roughly 60% of net primary production, confirming that they contribute significantly to the C input of the system. The overall carbon stored in the vineyard ( $152.1 \pm 7.1 \text{ t C ha}^{-1}$ ) was less than that of forests and some orchards primarily due to the lower amount of plant biomass. The soil represented by far the largest carbon storage in the vineyard, and changes in soil organic carbon and litter accounted for more than 75% of long-term carbon increase.

Further studies are needed to unravel the relative contribution of the grapevines and grasses to overall gross primary productivity and soil carbon storage, especially in the context of different management decisions and the increasing frequency of drought events in similar mountain environments.

**Keywords**

vineyard ecosystem, cover crops, organic management, eddy covariance, carbon balance, grapevine

biomass

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# 1 Introduction

Anthropogenic carbon dioxide (CO<sub>2</sub>) is the main contributor to the rising concentration of global greenhouse gasses (GHGs), which has led to global warming and the well-known climate change (CC) effects (Robertson et al., 2000; Carlson et al., 2017). Terrestrial ecosystems can partly mitigate this by assimilating CO<sub>2</sub> through photosynthesis and sequestering it into plant biomass and soil organic carbon (SOC).

While most studies in this area have focused on forests due to their high assimilation capacity and large standing carbon (C) stocks (Luyssaert et al., 2007), woody agroecosystems such as apple, peach, olive and palm orchards have also been reported as important sinks of CO<sub>2</sub> (Zanotelli et al., 2015; 2018; Navarro et al., 2008; Plénet et al., 2022). Indeed, while most studies consider arable lands as potential net CO<sub>2</sub> sources (Abdalla et al., 2013; Ceschia et al., 2010; Smith et al., 2007; Vleeshouwers & Verhagen, 2002), the potential to assimilate and store C in various long-term ecosystem compartments (trunk biomass, coarse roots, SOC, etc.) may tip the balance towards becoming net sinks.

The grapevine (*Vitis vinifera* L.) is one of the most cultivated perennial crops worldwide (FAOSTAT, 2021), largely due to its phenotypic plasticity which allows it to thrive in a wide range of climatic and soil conditions. Vineyard agroecosystems have also been reported to be net C sinks, but to widely varying extents ranging from 69 to 900 g C m<sup>-2</sup> yr<sup>-1</sup> (Guo et al., 2014; Marras et al., 2015; Pitacco and Meggio, 2015; Vendrame et al. 2019, Chiriaco et al. 2019; Table S1, Appendix B – Supplementary Material).

Determining if and to what extent agricultural land types may act as carbon sinks is important for understanding whether GHG mitigation goals can be met (Moinet et al., 2023). However, further insight into possible sources of variation requires a more holistic view of the C cycle. The carbon balance at the ecosystem level is determined by the difference between two large fluxes: the gross primary

productivity (GPP) as the photosynthetic input and the ecosystem respiration (Reco) as the output, with the difference being net ecosystem production (NEP). Additionally, to assess the value relevant to climate policy, the net ecosystem carbon balance (NECB; Chapin et al., 2006), we must consider lateral fluxes, such as harvest and organic fertilization, which are often high in agroecosystems. The C cycle is also affected by cultural practices and features such as irrigation, planting density, training system, canopy management, cover crops, and residue management, etc. (Longbottom and Petrie, 2015; Demestihias et al., 2017; Pardo et al., 2017; Buss et al., 2021), adding a layer of complexity with respect to unmanaged terrestrial ecosystems. Characterizing the spatiotemporal variation of different components of the C cycle might therefore be useful in altering management strategies to boost C sequestration and storage (Boss et al., 2021).

Vineyards with grass-covered alleys have been recently indicated as larger C sinks than their bare-soil counterparts (Tezza et al. 2019; Freibauer et al., 2004), highlighting the particular importance of floor management. While many farmers allow inter-rows to be colonised by spontaneous vegetation, cover cropping is also a widespread practice involving the cultivation of specific annual species between the main crop rows to provide several ecosystem services (Garcia et al., 2018; Payen et al., 2021, Guerra and Steenwerth, 2012). Cover crops specifically grown as an organic soil amendment aimed at increasing soil fertility and possibly SOC content are referred to as green manure (Longa et al., 2017). By boosting C sequestration of the system, each of these groundcover types may be regarded as a climate mitigation strategy (Smith et al., 2020; Buss et al., 2021, Fourie et al., 2007, Young et al., 2021), although to what extent is still under debate (Nistor et al., 2018; Novara et al., 2019).

The key question is how much of the C assimilated by a system is translated into long-term storage. While some studies (Janzen et al., 2022) maintain that maximising the net primary production (NPP, equivalent to plant growth) ultimately leads to high C storage, others (Zhou et al., 2021; Minasny et al., 2023) suggest that variables more loosely linked with assimilation, like the soil clay content, can have a

more relevant role in the stabilization of organic compounds and hence the long-term sink capacity.

More studies including a detailed description of NPP as well as standard factors involved in the C cycle are therefore needed to explore this further.

Currently, estimates of both seasonal C assimilation and partitioning within agroecosystems are sparse and uncertain. Only a few reports capture the spatiotemporal variation of nearly all C cycle components in specific perennial agroecosystems, such as apple and coffee (Zanotelli et al., 2015; Charbonnier et al., 2017). To our knowledge, such a complete study is still entirely missing for vineyards.

We selected an experimental organic vineyard where intercropping and green manure practices were applied and assessed its carbon balance, under the hypothesis that the site had a potential for carbon accumulation at least equivalent to if not greater than previously reported in vineyards. Furthermore, we aimed to improve understanding of its carbon cycle by quantifying the magnitude of different pools and the variation of C fluxes throughout a growing season. By focusing on the NPP contribution of individual ecosystem components, we explored the immediate fate of assimilated carbon and the relative importance of ground vegetation.

## 2 Material and Methods

### 2.1 Site description

The study was conducted in a mountain vineyard in Caldaro, South-Tyrol (46° 24' N, 11° 15' E, 325 m a.s.l.) in the North of Italy, on the West-facing slope of the Adige Valley. The experimental vineyard was 0.85 ha in size, with homogenous terrain and a 5% slope. The surrounding area was also predominantly used for grapevine cultivation. The Köppen-Geiger climate classification is Cfb (temperate oceanic). The 30-year average temperature and precipitation (1991-2020, provided by nearby Laimburg Research Center) are 12.0 °C and 829.5 mm year<sup>-1</sup> respectively. The soil texture (15-30 cm depth) is sandy loam (USDA classification), with 57.4 ± 1.2% sand, 28.4 ± 0.4% silt, 14.3 ± 0.8% clay, 2.44 ± 0.08% soil organic matter and a high content of stones (49.6 ± 1.1% of total weight). Two white *Vitis vinifera* L. cultivars (Chardonnay and Sauvignon blanc grafted on SO4 rootstock) covering similar portions of the vineyard surface were planted in 2008 and managed according to the EU guidelines of organic farming from 2009 onwards. The usage of the site for viticulture predates the current plantation. Rows were North-South orientated with inter-rows 2 m wide and inter-vine spacing of 0.8 for Sauvignon blanc (5882 vines ha<sup>-1</sup>) and 0.75 m for Chardonnay (5667 vines ha<sup>-1</sup>). Vines were trained on a vertical shoot positioning (VSP) system and cane-pruned (single Guyot).

The growing season of 2021 (mid-April till mid-November) was characterized by many rainfall events of variable intensity, occurring primarily during May, July and the beginning of August. Given that June and mid-September were relatively dry periods, plants were irrigated twice a week via a drip irrigation system from June until mid-July and once in September, comprising a total water volume of 500 m<sup>3</sup> ha<sup>-1</sup>. Soil water content (SWC) ranged from 8 to 26% over the course of the growing season and responded to rainfall and irrigation. The mean daily Tair (air temperature) during the growing season was within the



range of 11 – 23 °C. The highest temperatures in the season were observed in the period between June and August, with mean  $T_{air} > 22$  °C and VPD (vapour pressure deficit)  $> 8$  hPa, while the highest values of  $T_{air}$  and VPD were registered in June, in coincidence with the lack of precipitation. See Fig. S1 (Appendix B - Supplementary Material) for more detail.

The vineyard floor was vegetated and green manure planted in alternating inter-rows: while one inter-row was tilled (5 cm depth) and sown with a mix of grasses and legumes, the adjacent one was allowed to be recolonized by resident vegetation. The latter was mown three times during the summer and the former once in Autumn. The vegetation under the vine row (40 cm wide) was cleared mechanically at each mowing event. See Table S2 (Appendix B – Supplementary Material) for a list of species identified.

Annual fertilization comprised an average of 0.125 kg per plant of organic fertilizer (plant material partially converted into fungal biomass; Agrobiotec<sup>®</sup>, Austria). The grapevine growing season was mid-April to mid-November, 2021. Budburst occurred in early April, 50% flowering by the end of April, veraison in early August and harvest in mid-September.

## 2.2 Net Ecosystem Exchange and meteorological measurements

The net ecosystem exchange (N.E) was measured by the eddy covariance (EC) technique. The equipment included a 3D ultrasonic anemometer (Gill R3-50, Gill Instruments, Lymington, UK) and an enclosed-path infrared (IR) gas analyzer (LiCor LI-7200), set at 6 m above ground. Air samples were taken through an insulated steel tube of 4 mm in internal diameter and 0.75 m in length at 0.2 m from the anemometer. The airflow rate was 10-12 L min<sup>-1</sup>, as provided by the flow module (7200-101, Li-Cor, Lincoln, NE, USA). Raw values of CO<sub>2</sub> and H<sub>2</sub>O concentration and 3D wind speed components were measured at 20 Hz and the resulting fluxes were computed and logged by a personal computer every 30 minutes using the Eddysoft software (Kolle and Rebmann, 2007, Mauder et al., 2008),). Solar radiation

components were measured by a CNR1 net radiometer (Kipp & Zonen, Delft, Holland); air temperature and relative humidity by a CS215 probe (Campbell Scientific Incorporated, CSI, Logan, Ut, US); rainfall by a tipping bucket rain gauge (RAIN-O-MATIC, Pronamic, Silkeborg, DK), and soil water content by two TDR sensors (CS616, CSI) installed horizontally at 15 and 50 cm depth. Fluxes and meteorological data were measured every 10 s, averaged across 30 min and logged by a CR3000 (CSI) datalogger. Continuous measurements were taken during the growing season.

Gap filling and partitioning of NEP into GPP and Reco - see Eq. 7 - was done using the 2015 version of the REddyProcWeb online provided by the Max Planck Institute of Jena (Wutzler et al., 2018), which implements algorithms in the PV-wave programming language. Partitioning was performed using nighttime (Reichstein et al., 2005) and daytime (Lasslop et al., 2010) methods, each with and without  $u^*$  selection.

The energy balance closure of the EC tower revealed that the turbulent flux (LE+H) was 73.9% of all available energy (Rn-G). The flux footprint (area comprising 90% of the flux source) was calculated following Kljun et al. (2015) and had an oval shape centred in the tower, oriented south-east. The footprint length and width were  $\pm 200$  m and  $\pm 100$  m, respectively. The footprint and energy balance closure are shown in Fig. S2, Appendix B – Supplementary Material.

## 2.3 Total and heterotrophic soil respiration

Within the flux footprint, three experimental blocks were designated for each grapevine cultivar. To assess total soil respiration (Rs) four stainless steel collars (20 cm diameter, 10 cm height) were installed in the soil to a depth of 7 cm in each of the six experimental blocks; two in the vine row and one in the middle of each inter-row to either side at 1 m from the vines. To assess heterotrophic respiration (Rh) a total of six additional collars of the same dimensions were installed in interrow plots of 0.6 m diameter

(as per Panzacchi et al., 2012) that had been trenched by digging to a depth of 50 cm and inserting a non-fabric polyethylene material to prevent further ingrowth of roots. This was done 45 days before relevant flux measurements. In this way, CO<sub>2</sub> efflux from the soil was attributed to microbial respiration only (Kuzyakov, 2006; Subke et al., 2006; Tomè et al., 2016). A schematic representation of the layout and structure of the blocks is shown in Fig. S3 (Appendix B - Supplementary Material).

A 20 cm survey chamber (LI-8100-104, Li-Cor) in conjunction with an IR gas analyser (Li-8100, Li-Cor) was used to take spot readings on all collars every three weeks, starting in May. Vegetation inside the collars was cut down to approximately 1 cm above ground level prior to measurement of Rs and manually removed in trenched plots. The duration of each soil respiration measurement was 150 s in total, consisting of 30 s of pre-purging, 90 s of data collection and 30 s of post-purging. Fluxes were calculated automatically using the LI-8100 File Viewer Version 3.0.0 (Li-Cor, Lincoln, NE, USA), applying an exponential fit (Zhao et al., 2018) and excluding the first 10 s of data, which was considered the mixing period. Soil temperature was measured using an auxiliary temperature sensor at 10 cm depth.

Since measurements were not continuous across the season, gap-filling was done by fitting flux data to two widely used models for predicting soil respiration based on local air temperature: the Q<sub>10</sub> (Van't Hoff, 1898; Eq.1) and the Lloyd-Taylor models (Lloyd and Taylor, 1994; Eq. 2). To account for the effects of soil water content (SWC), a modificative term based on a simple saturation model (Bunnell et al., 1977; Hanson et al., 1993, Eq. 3) was included. This describes soil respiration as being multiplicatively dependent on SWC and assumes that the sensitivity to temperature is independent of SWC (Reichstein et al., 2002).

$$R = R_{ref} * Q_{10}^{\frac{T_s - T_{ref}}{10}} \quad [1]$$

$$R = R_{ref} * \exp(E_0 * (0.0178507 - \frac{1}{T_s + 46.02})) \quad [2]$$

$$R = f(Ts) * \frac{SWC}{SWC + SWC_{1/2}} \quad [3]$$

Here R refers to respiration (Rs or Rh) and Rref to the respiration at the reference temperature (Tref) of 10 °C.

The four resulting models were compared using R-squared ( $R^2$ ), Mean Absolute Error (MAE), Root Mean Squared Error (RMSE) and Akaike's Information Criterion (AIC), and the best one selected to produce a continuous time series of soil respiration with a resolution of 30 min for the growing season based on meteorological data recorded as described in Section 2.2. All modelling was performed in R, using the lm base function for linear regressions and the nls package for non-linear fits (R Core Team, 2021).

Rs data recorded during the growing season showed that the most suitable method for gap-filling data was the Lloyd Taylor model, including the SWC term only for plots located in the vine row. The Rs of the vineyard was then calculated as a weighted average of the modelled fluxes based on the widths of each grass cover type per block (Tezza et al., 2015), which were 0.4 m and 1.6 m for the vine row and inter-row respectively.

## 2.4 Biometric measurements

Biometrically-assessed NPP (NPPbiom) was the sum of C accumulated in the different ecosystem components during the season. This was estimated using the difference between biomass assessed on consecutive dates (Clark et. al., 2001). The considered components of NPPbiom (see Eq. 11) were the following: NPPI (vine leaves), NPPf (fruit), NPPc (cane biomass), NPPg<sub>AG</sub> (above-ground grassed alley biomass), NPPg<sub>BG</sub> (below-ground grassed alley biomass), NPPw<sub>AG</sub> (above-ground permanent woody biomass, i.e., the trunk), NPPw<sub>BG</sub> (below-ground permanent woody biomass, i.e., coarse roots) and NPPfr (grapevine fine roots). As in Zanotelli et al. (2013), root exudates were not measured for practical

reasons. Non-CO<sub>2</sub> volatile emissions were assumed to contribute negligibly to NPP (Arneth et al., 2011; Malhi et al., 2009).

To assess grapevine biomass, a combination of measurements for the selected vines and destructive sampling of nearby vines was performed every  $21 \pm 7$  days, as in Zanotelli et al. (2013). Temporary vegetative biomass (canes and leaves) was calculated using allometric equations based on the length of individual shoots. To establish the site-specific parameters ( $a$  and  $b$  in Eq. 4), five randomly selected shoots per cultivar were harvested from separate vines adjacent to the experimental blocks. The length of each ( $L$ ) was measured from base to apex and the total leaf, cane and fruit dry weight ( $DW$ , g) determined after drying at 70 °C to constant weight. Cane and leaf  $DW$  were fitted against  $L$  by non-linear regression based on all measurements for each cultivar throughout the season. A power relationship was used (Miranda et al., 2017), showing good fits (adjusted  $R^2$  values  $>0.87$ ). The standing biomass of the canes ( $SBC$ ) and leaves ( $SBL$ ) per vine were calculated as follows, using the average shoot length ( $L_{avg}$ ) and number of shoots ( $N_s$ ) per plant:

$$SBL, SBC(t) = a * L_{avg}(t)^b * N_s(t) \quad [4]$$

$N_s$  was counted for all vines in each block (total of 18 vines for Sauvignon blanc and 21 for Chardonnay), while  $L_{avg}$  was determined from the length of all individual shoots of the same two designated vines per block at each date (on average  $54 \pm 2$  shoots measured per cultivar). Fruit standing biomass ( $SB_f$ ) for each plant was calculated using the average cluster  $DW$  of the shoots harvested at each date multiplied by the average number of clusters per vine (counted once for all vines per block after shoot thinning at the beginning of the season).  $NPP_i$ ,  $NPP_c$  and  $NPP_f$  were calculated in  $g C m^{-2}$  using the increment in  $SB$  together with the carbon concentration of the respective organ (see Section 2.6) and the ground surface area per vine ( $A$ ; the product of inter-row and inter-plant distance).

$$NPP(t) = \Delta SB(t) * [C] * A^{-1} \quad [5]$$

NPP of the ground cover was measured destructively by mowing. One quadrant of 1 m<sup>2</sup> and one of 0.16 m<sup>2</sup> per block were marked in the inter-row and in the vine-row respectively (Fig. S3B, Appendix B - Supplementary Material) and all vegetation > 1 cm above ground within collected every 21 ± 7 days. Biomass was dried at 70 °C to a constant weight for DW measurement. This DW represented the shoot growth per quadrant between measurement dates ( $\Delta SB_{gAG}$ ), and was converted to  $NPP_{gAG}$  as per Eq. 5, with A substituted by the quadrant area. In this case, the general literature value of [C] as 45% of DW (Schlesinger, 1997) was used.

The woody and below-ground components of the grapevine biomass were also measured destructively, by excavating three sequential vines of each cultivar (replicates A-C in Fig. S4, Appendix B – Supplementary Materials) at the end of the season from two different rows outside of the experimental blocks. The six vines were each extracted by hand within a surface area of 0.4 m x 0.4 m centred on the vine trunk and to a depth of 1 m, keeping the root system as intact as possible. They were then divided into one year-old canes (SBc), two-year-old canes, trunk and roots (coarse and fine), and the DW of these components measured after drying. The trunk diameters were measured at 10 cm above the ground with vernier calipers, taking the average of two perpendicular measurements following the ICOS protocol for ancillary vegetation measurements (Gielen et al., 2018). This and SBc were compared with values measured for all vines in the experimental blocks at the end of the season. The average dry weight of the vine trunks was then divided by the age of the vines (14 years) to obtain an estimate of the average annual  $\Delta SB_{wAG}$ , which is assumed to be equal to the  $\Delta SB_{wAG}$  of each growing season. This was converted to  $NPP_{wAG}$  as per Eq. 5.  $NPP_{wBG}$  was similarly obtained using the average total coarse root (>2 mm in diameter) biomass.

The growth of temporary underground components ( $NPP_{fr}$  and  $NPP_{gBG}$ ) was estimated using the determined fine root biomass in combination with turnover rates reported in literature, assuming that the fine root production is in equilibrium with mortality rate (Gill & Jackson, 2000):

$$NPP_{fr}, NPP_{gBG} = [C] * \text{fine root biomass} * \text{turnover rate} \quad [6]$$

For grapevines, the turnover rate used was 1 year<sup>-1</sup> (Agnelli et al., 2014; Amendola et al., 2017). For the ground cover, a rate of 1.3 year<sup>-1</sup> was used based on the study by Leifeld et al. (2015) and the mean annual temperature of our site. The fine root biomass in Eq. 6 was the sum of the directly extracted fine root DW and of the value calculated based on the root density distribution, as described in Section 2.6. In the case of the herbaceous ground cover, the extracted root biomass was determined at the start of the season by excavating six grassed alley patches of 0.04 m<sup>2</sup> to a depth of 5 cm depth, after which roots were washed and separated from the shoots before measuring DW.

## 2.5 Flux Partitioning

Based on the measurements described in the above sections, ecosystem fluxes were disentangled as follows:

First, observed NEP from the EC measurements was partitioned into its relative components (see Section 2.2).

$$NEP = GPP - Reco \quad [7]$$

Ecosystem respiration was then separated into above- and below-ground sources using total soil respiration measurements (Eq. 8) and Rs broken down into its autotrophic (Ra) and heterotrophic components in Eq. 9 using the measured values of Rh.

$$Reco = Ra_{AG} + Rs \quad [8]$$

$$Reco = Ra_{AG} + Ra_{BG} + Rh = Ra + Rh \quad [9]$$

Where  $Ra_{AG}$  and  $Ra_{BG}$  are the above- and below-ground autotrophic respiration respectively. The latter was calculated for each soil respiration control plot by subtracting the mean  $Rh$  across all trenched plots at that date from the respective  $R_s$  value.

Considering that NPP is the difference between GPP and  $R_a$ , a mass balance-based value (NPPflux) was calculated using EC and soil fluxes:

$$NPPflux = GPP - (Reco - Rh) = NEP + Rh \quad [10]$$

The contribution of different NPP components to the total NPPflux was estimated using the biometric methods described in Section 2.4.

$$NPPflux \approx NPPbiom \quad [11]$$

$$= NPPl + NPPf + NPPc + NPPw_{AG} + NPPv_{BG} + NPP_{FR} + NPPu_{AG} + NPPu_{BG}$$

## 2.6 Soil determinations and elemental analysis

At the end of the season, a series of soil samples at various depths and positions relative to each vine was taken for analysis of soil properties and root density (Böhm, 1979). First, a trench was dug using a mechanical digger to a depth of 1 m alongside the selected vines in each of the two rows. Three depth profiles were then made for each vine using five intervals of 20 cm to a maximum depth of 1 m. Position 1 was located directly at the base of the trunk, position 2 along the vine row half-way between the selected vine and the next, and position 3 in the middle of the inter-row, 1 m from the vine trunk, see Fig. S4 (Appendix B – Supplementary Materials) for more details. At each depth interval a small amount of soil was excavated and the volume of the hole estimated by placing a mesh bag inside and filling it with marbles until they were level with the top of the hole, as done by Rodeghiero and Cescatti (2005). The weight of the marbles was then measured and converted to volume using separate linear regressions to determine the volume and weight of each marble. The soil excavated at each depth was



collected and dried at 70°C to constant weight. Each dried sample was weighed and sieved through a 2 mm mesh, allowing separation into stone, fine soil and coarse organic material fractions, which were then weighed separately. The coarse organic fraction was separated into coarse vine roots, fine vine roots, fine grass roots and other organic matter. Bulk density of each sample was calculated using the total dry weight (Blake and Hartge, 2018), including stones.

Calculation of additional vine root biomass based on the soil samples was done by interpolation of the total root densities ( $\text{g DW cm}^{-2}$ , down to 1 m in depth) across different surface positions. The assumptions made were that there were linear relationships between points and that root density within each zone was homogenous, implying that there was no variation along the inter-row and that the root distribution was uniformly bi-symmetrical (Kozma, 1977 in Smart et al., 2006). This calculated biomass was then added to that of the extracted root system (fine and coarse roots respectively).

The fine soil fractions - along with tissue samples of the cane, trunk, leaves, and coarse roots of the extracted vines - were crushed using a steel ball mill (Retsch Mixer Mill MM 400) at 25 Hz for 30 s. C and N concentration (%) were determined by elemental analysis using a FlashEATM 1112 Elemental Analyzer (Thermo Fisher Scientific, Germany). Soil samples were acidified prior to analysis, and the concentration values converted to SOC/nitrogen density ( $\text{g C L}^{-1}$ ) using the mass of fine earth per unit of total sample volume. The stones were considered to not contain any carbon. The total SOC content per  $\text{m}^2$  (considering the maximum depth of 1 m) at each surface position was calculated by integrating the SOC density of each layer multiplied by its thickness (0.2 m) across all depths.

## 2.7 Statistical analysis and uncertainty estimate

In this study, uncertainty is always indicated as the SEM (Standard Error of the Mean). With regards to the EC-measured fluxes, SEM was assessed based on the outcomes of the four different calculation

methods (nighttime or daytime partitioning with and without  $u^*$  selection). In the case of soil respiration fluxes, uncertainty was due to the spatial heterogeneity between plots as well as the goodness of fit of the soil respiration models. This was reflected in the standard errors of the model parameters, which were used to generate different respiration flux outputs from which the mean and SEM were calculated. For the biometric measurements and soil samples, uncertainties were mainly due to random errors and spatial/inter-plant heterogeneity.

When calculations were performed using mean values (X and Y in the example), the SEM of the result (Z) was calculated according to error propagation theory (Taylor, 1997), as shown below.

If  $Z = X + Y$  (e.g., for the total of a series of observations):

$$SEM_Z = \sqrt{SEM_X^2 + SEM_Y^2} \quad [11]$$

If  $Z = X \cdot Y$  (e.g., calculating carbon content from DW and [C]):

$$SEM_Z = Z * \sqrt{\left(\frac{SEM_X}{X}\right)^2 + \left(\frac{SEM_Y}{Y}\right)^2} \quad [12]$$

When a mean was multiplied using another value with no errors, the corresponding SEM was simply multiplied by the same value.

## 3 Results

### 3.1 Eddy covariance

Over the course of the growing season, the total NEP (-NEE) of the vineyard was  $246 \pm 54 \text{ g C m}^{-2}$ , indicating that the vineyard acted as a sink of C. Total GPP and Reco were  $2409 \pm 35 \text{ g C m}^{-2}$  and  $2163 \pm$

88 g C m<sup>-2</sup>, respectively. The fruit harvest removed 83 ± 3 g C m<sup>-2</sup> on average, resulting in a NECB of 163 ± 54 g C m<sup>-2</sup>. To further understand the pattern of the C balance components, we analysed them on a daily (Fig. 1A) and monthly (Fig. S5, Appendix B – Supplementary Material) basis. From April to August, NEE was negative due to GPP being higher than Reco, but from September to November presented positive values, indicating a net release of CO<sub>2</sub>. The lowest NEE values (maximum production) were achieved in August. The highest GPP values were registered in July and August, followed by May, June and October. It can be observed that there were two distinct dips of GPP and Reco in June and September, hence less negative NEE daily values. These values are in coincidence with a dry (low SWC) and hot period (high VPD, especially in June). After this period, GPP achieved the highest daily values in July and August.

### 3.2 Soil respiration

Rs presented relatively high values that followed the seasonal trend of Reco, demonstrating that it was the major component of ecosystem respiration. The highest values of Rs were recorded in July and August and the lowest in June (coinciding with the hot and dry period) and September and October (due to low temperatures), as shown in Fig. 1B. Total Rs of the growing season was 1807 ± 99 g C m<sup>-2</sup>. The total Rh (Fig. 1C) of the season was 947 ± 154 g C m<sup>-2</sup> leaving an R<sub>aBG</sub> of 861 ± 183 g C m<sup>-2</sup> (Fig. 1D).

### 3.3 Biometric NPP and C storage

As an overview of carbon storage partitioning within the ecosystem, Fig. 2 shows partitioning of carbon stocks present in the vineyard just prior to harvest. The [C] of the grapevine organs was within the range of 40.2 - 45.8% (see Table S3, Appendix B – Supplementary Material, for information on DW, [N] and [C]). The majority of C within the grapevines was contained in the permanent woody biomass: 554.5 ± 63 g C m<sup>-2</sup>, of which 65% was coarse roots. Considering the age of the vines, this corresponded to an

average seasonal accumulation of  $39.6 \pm 4.5 \text{ g C m}^{-2}$  in the woody tissues. Including fine roots, the below-ground biomass of the vines was on average 53.2% of the plant total, equivalent to  $450 \pm 66.8 \text{ g C m}^{-2}$ . Total NPPfr was  $91.2 \pm 37.3 \text{ g C m}^{-2}$ . The NPP of the annually-produced shoots was  $201.9 \pm 1.7 \text{ g C m}^{-2}$ , of which 41% was fruit biomass and the remainder was vegetative growth.

The six vines extracted destructively at the end of the season had a mean trunk diameter of  $3.2 \pm 0.3 \text{ cm}$ , which matches that of the 39 experimental block vines ( $3.2 \pm 0.1 \text{ cm}$ ). Similarly, there was overlap between the mean DW of canes  $\text{vine}^{-1}$  at the end of the season for the destructively harvested and allometrically assessed vines ( $189.4 \pm 29.8 \text{ g DW}$  and  $226.5 \pm 12.3 \text{ g DW}$  respectively), with the latter being slightly higher.

NPPbiom for the growing season was  $841 \pm 59 \text{ g C m}^{-2}$ . Of this, 45.7% can be attributed to below-ground NPP (Fig. 3; Table S4, Appendix B – Supplementary Material). The grass/green manure shoots accumulated a larger amount of C over the course of the growing season than that of the vine shoots produced in the same timeframe ( $231.8$  vs.  $201.9 \text{ g C m}^{-2}$ ). Most of the groundcover roots were in the upper 10 cm of soil and represented a much greater biomass at any given point in the season than the shoots. However, the seasonal NPP of the roots was only slightly larger. Grapevine leaf and cane growth occurred continuously from budburst in April until October, with the maximum growth rates in June, after which fruit became the dominant sink. Fruit growth began in mid-April and increased in rate after flowering near the end of May, with a particularly high accumulation of carbon in August, which comprises the period between veraison and harvest.

### 3.4 Soil C and N content

The mean soil bulk density in the vineyard was  $2061.7 \pm 42.0 \text{ g L}^{-1}$  (stones included), with the lowest values generally occurring in the surface layer of the soil. The carbon content of the fine soil fraction

showed decreasing values with depth (Fig. 4), with means of  $20.8 \pm 2.7 \text{ g C L}^{-1}$  at 0-20 cm down to  $7.5 \pm 0.6 \text{ g C L}^{-1}$  at 80-100 cm. The average total soil carbon (to a depth of 1 m) was  $14.1 \pm 0.7 \text{ kg C m}^{-2}$ , representing the largest ecosystem C pool, and that of nitrogen was  $1.2 \pm 0.1 \text{ kg N m}^{-2}$ . The highest concentrations of C and N (29.5% and 26.4% of total, respectively) occurred in the upper 0-20 cm layer of the soil.

### 3.5 Seasonal C flows

The total fluxes for the growing season were combined to give an overview of the flow of C-cycle components of the agroecosystem (Fig. 5), including lateral fluxes such as organic fertilizer input (OF) and harvest. In addition, this graph shows derived seasonal values based on the directly-measured fluxes mentioned previously:  $R_{aBG}$  was  $861 \pm 183 \text{ g C m}^{-2}$ , comprising most of the total ecosystem  $R_a$  ( $1216 \pm 177 \text{ g C m}^{-2}$ ), whereas the remainder allocated to  $R_{aAG}$  ( $355 \pm 133 \text{ g C m}^{-2}$ ) was relatively minor. The NPPflux for the growing season was  $1152 \pm 163 \text{ g C m}^{-2}$ , as per Eq. 10. This was larger than NPPbiom by  $352 \text{ g C m}^{-2}$ . The NECB remaining after subtracting the harvested fruit C from the NEP was  $163 \pm 54 \text{ g C m}^{-2}$ .

## 4 Discussion

Here, we are reporting for the first time the whole carbon cycle of a grassed vineyard, as well as the spatiotemporal evolution of its components.

### 4.1 Overall fluxes

The seasonal net ecosystem production (NEP) of the vineyard, although positive, was toward the low end of the reported range of  $69 - 900 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Table S1, Appendix B – Supplementary Material). For

comparison, an apple orchard in northern Italy presented an NEP of roughly  $480 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Zanotelli et al., 2015; 2018) and forests in a similar latitude and climate may accumulate up to  $600 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Luyssaert et al. 2007), indicating higher rates of carbon storage increase. NEP was further reduced by 33.7% due to the export of harvested fruit biomass, a feature particular to agroecosystems.

Conversely, we observed much larger gross primary production and ecosystem respiration (GPP and Reco) in our study than previously reported in vineyards based on whole-year measurements (Marras et al., 2015; Vendrame et al., 2019). Our values are also nearly double those of the aforementioned forests and apple orchard (Luyssaert et al., 2007; Zanotelli et al., 2013). This, in combination with the low NEP, shows high assimilation at our site which was mostly offset by closely matching C emissions. A similar magnitude and pattern of fluxes was reported by Kirschbaum et al. (2020) for dairy pastures and by Abdalla et al. (2013) for grasslands, indicating that our vineyard behaved more like a managed grassland than other woody agroecosystems.

The magnitude of these eddy covariance (EC) fluxes was validated against two criteria: the total soil respiration ( $R_s$ ) and the EC energy balance closure.  $R_s$  made a significant but reasonable contribution (83%) to Reco (Fig. 1B), indicating via independent measurement that the EC method is unlikely to be at fault. The energy closure of 75.2% was also within the 20-30% discrepancy commonly observed in surface energy budget measurements (Wilson et al., 2002). We can therefore assume, given the data available, that the size of the fluxes is due to unique characteristics of the ecosystem.

## 4.2 Fate of sequestered C

The input of C to the system can be better understood by examining the partitioning of net primary production (NPP), which represents half of GPP. Our results show that the grassed alleys are responsible for 60.4% of biometrically assessed NPP (Fig. 3; Fig. 5), likely due to a relatively wet summer period and

the open canopy structure and North-South orientation of the vine rows, which together exposed the vineyard floor to non-limiting growth conditions. This supports the grassland-like behavior of the agroecosystem observed in the EC fluxes.

However, most NPP was then lost as heterotrophic respiration (Rh), which was the prevalent component of Rs (52%). This has been previously reported for woody agroecosystems (Zanotelli et al., 2013), and is likely due to the high input of litter material from pruning and mowing. The proportion of litter material lost as Rh in a year depends on its quality, which comprises carbon chemistry and N content (Prescott, 2010). The woody grapevine canes, which had the highest C:N ratio amongst litter components (Table S3, Appendix B – Supplementary Material), have been reported to lose approximately 40% of material in a year (Brunetto et al., 2018), as opposed to ~50% in the case of grapevine leaf litter carbon (Tagliavini et al., 2007). These decomposition rates can of course vary based on climate conditions and management (such as the shredding of pruned material in our vineyard), but nonetheless imply a yearly buildup of carbon as detritus.

Regarding cover crops, Brunetto et al. (2011) found a mixture of ryegrass and white clover (respectively low C:N ratio and lignin content) to lose nearly 80% of C within only 16 weeks on a vineyard floor in Italy after cutting, indicating that this material is rapidly decomposed and a relatively small portion goes to long-term storage compared to grapevine litter. The input of nitrogen into the litter layer through N-fixing green manure cultivated at our site (mainly *Vicia* sp.) may also have contributed to the high Rh flux by making the carbon present more available to heterotrophic microorganisms. Therefore, as suggested also by recent meta-analyses (Gross and Glaser, 2021; Jian et al., 2020; Young et al., 2021), while cover crops and green manure lead to an increase in the C turnover in agroecosystems, their contribution to increasing long-term storage cannot be generalized to all sites and management systems.

The NPP which is not lost as  $R_h$  within the year contributes to net ecosystem carbon balance, which can be attributed to changes in three C pools: soil organic carbon (SOC), undecomposed litter and long-term living biomass. The last compartment includes only the woody grapevine organs, assuming yearly average herbaceous biomass to be relatively small and at a steady state. Our study confirms this to be the largest vine C reservoir at any point in the season (Zhang et al., 2021; Miranda et al., 2017; Brunori et al., 2016; Williams et al., 2020). The annual biomass increment, while accounting for only 4.7% of NPP<sub>biom</sub>, was equivalent to 24.2% of the net ecosystem carbon balance, showing the significance of this long-term pool from which little C is lost during the lifespan of the vines.

The combined SOC sequestration rate and litter buildup can be considered the primary long-term sink of the vineyard in 2021, accounting for the remaining 75.6% of the net ecosystem carbon balance. This too is likely a consequence of management practices such as pruning and mowing, which transfer almost all the carbon in the temporary biomass pool to the litter layer.  $R_s$  at our site was roughly three-fold greater than reported for other vineyards with higher SOC concentrations and lower soil bulk densities (Carlisle et al., 2006; Vendrame et al., 2019; Volff et al., 2018), which should be conducive to increased  $R_s$ . This implies that much of the respired C came from litter material that was degraded before forming stable SOC.

Despite the rapid first-phase decomposition of the labile litter fraction represented by groundcover, Cotrufo et al. (2013) states that high-quality litter is conducive to increased SOC storage in the long term. Consequently, although a much higher fraction of the groundcover litter is lost as  $R_h$  than that of the vines, a higher portion of that which remains may contribute to SOC. It therefore seems likely that the litter buildup is primarily due to grapevine pruning material (and to a lesser extent leaves), while the herbaceous species (especially N-fixing cover crops) contribute more to SOC sequestration, especially since they represented most of the system's NPP.



It is also noteworthy that fine root production was estimated to account for roughly half the grass cover NPP, which likely increased the SOC especially in the uppermost soil layers (Fleishman et al., 2021), where most of the SOC at our site was located. The grapevine fine roots contributed much less C overall but were present at greater depths, where SOC has a longer residence time (Janzen et al., 2022). The higher grass root turnover may reduce decomposition of vine roots in deeper horizons by providing preferentially degraded substrates (Agnelli et al., 2014), in this way supporting long-term storage.

### 4.3 Vineyard C storage

The total C stock represented by the grapevines is influenced by the C concentration of each organ. To our knowledge, few studies assessing whole-grapevine C storage have measured this, relying mostly on literature. Our findings are in line with those of Zhang et al. (2021), with the exception lower fruit C concentration. It is noteworthy that our value for the vine trunk ( $44.5 \pm 0.4\%$ ) was slightly higher than that determined by Munalula and Meincken (2008, 43.7%), which has been cited in literature reviews related to vineyard C storage (Nistor et al., 2018).

Aside from this, vineyard planning and management plays a prominent role in the grapevine C stocks per ha by influencing the average vine biomass and the planting density (vines  $\text{ha}^{-1}$ ). At our site, vine biomass was like that of other vertically-trained vineyards of various cultivars (Miranda et al., 2017; Scandellari et al., 2016), and was fairly low in comparison with more high-vigour systems such as the Geneva Double Curtain (Morande, 2015). This may be offset by the fact that smaller vines allow for higher planting density. For example, although the vines studied by Scandellari et al. (2016) had a higher average biomass, the planting density was lower (3077 vines  $\text{ha}^{-1}$ ), resulting in  $7.3 \pm 0.5 \text{ t DW ha}^{-1}$  as opposed to  $12.5 \pm 0.7 \text{ t DW ha}^{-1}$  in our study. This indicates that although other variables like cultivar, soil and climate may factor into vine biomass, the chosen training system and subsequent planting density and pruning method are dominant in determining long-term vine C stocks.

To put this in perspective, it is important to consider the vineyard living biomass C pool in relation to that of its soil. Of the total C stored in the vineyard ecosystem ( $152.1 \pm 7.1 \text{ t C ha}^{-1}$ ; see Table S3, Appendix B – Supplementary Material), 92.7% was contained in the upper 1 m of soil (not including roots) and the remainder in living plant biomass. The latter is reduced to 3.6% of the total if one considers only the permanent woody organs. Although these proportions can differ in other vineyards such as that of Zhang et al. (2021) based on training system and soil type, soil is clearly the most important C pool agroecosystems like vineyards where the living biomass pool is relatively low. In contrast, a mature citrus orchard in China was found to have a similar level of soil C, but higher total C due to the above-ground biomass (Wu et al., 2014). The same applies to European forests, where the mean computed SOC was equivalent that of our vineyard (CODEF model, de Vos et al., 2015), but the living biomass was estimated to be roughly 10-fold greater than the permanent biomass of our vines (Thurner et al., 2014). This shows firstly that the vineyard can maintain high SOC levels equivalent to natural ecosystems, and secondly that management with a focus on long-term C sequestration should always consider the impacts on soil first and foremost.

Because the strong groundcover activity, one might expect the SOC content in our vineyard to be similar to that observed in grasslands, which are commonly used as benchmarks for agricultural sustainability. However, these were reported to contain  $41.1 \text{ t C ha}^{-1}$  more within first 1 m of soil than our site (Glover et al., 2010), especially in the topsoil, where the SOC was >50% higher (Moll-Mielewczik, et al., 2023) for grasslands in a similar climate. This may be because the vineyard has not yet reached its 'effective' storage capacity, defined as the maximum amount of C that the soil can hold given its physio-chemical properties and the environmental constraints on C stabilization (Stewart et al., 2007). Grassed hazelnut orchards in Italy have been found to take > 35 years to regain pre-cultivated grassland SOC levels (Pacchiarelli et al., 2022). Our site, however, was cultivated with grapevines prior to the current plantation, and given the similarity of SOC stocks with other perennial agroecosystems as well as the

close match between Reco and GPP, it seems more likely that it is a question of management defining the effective storage capacity of the soil rather than saturation not having been reached. This is supported by the fact that another study performed in the Marche region of Italy (Agnelli et al., 2014) found SOC close to ours in grassed vineyard rows. Management-influenced factors such as a lower root density, shallower rooting depth, tillage, mowing/cutting, and a limited area of the vineyard floor covered by the green manure could all plausibly contribute to a lower SOC.

#### 4.4 Limitations and uncertainties

In terms of the seasonal pattern of fluxes, the mowing events which occurred in May, June and July of 2021 to facilitate the movement of workers may have had particular relevance. It follows that mowing would cause a sharp decrease in GPP, followed later by a rise in Reco as the material decomposes. We cannot exclude that this contributed to the seasonal pattern of C fluxes shown in Fig. 1, but further studies such as that of Legesse et al. (2022) would be needed to disentangle the effects of management on the groundcover from those of environmental variation. Indeed, the two dips in both GPP and Reco observed during the measurement period (in June and August-September, respectively) also coincided with periods of drought and high temperatures. These adverse conditions are likely to have affected the grasses most, given their shallower roots and the lack of irrigation in the inter-row. SWC has been documented as a primary controller of above-ground biomass and C sequestration in grasslands (Hu et al., 2018; Zhang et al., 2016), which is in line with the reduced NEP observed during the drought periods in 2021.

It is also worth noting the discrepancy between the two measures of this (NPP<sub>biom</sub> and NPP<sub>flux</sub>) in our study. While a portion of this is likely due the fact that root exudates and non-CO<sub>2</sub> volatiles were not measured, leading to NPP<sub>biom</sub> being an underestimate (Zanotelli et al., 2013), uncertainties in estimating R<sub>h</sub> may also have contributed. The experimental method for separating R<sub>h</sub> from R<sub>s</sub> by

trenching is known to have drawbacks including modified soil water content (Kuzyakov, 2006), which together with root decomposition can lead to Rh being overestimated, especially during drought periods (Savage et al., 2018). The effects of SWC could not be included in the gap-filling process of Rh, likely because sensors were located in the vine row, which was not representative of inter-row or trenched plots. During dry periods, this would have led to an overestimation of Rh and therefore NPPflux. Both issues emphasize the importance of precise and local (plot-level) environmental data collection when using chamber-based measurements to represent a spatially heterogeneous area.

It is also important to consider errors inherent in the biometric measurements. As in many studies, the method used for assessing below-ground NPP was more subject to error than for that of the above-ground components. The sample size for total root biomass (both vines and grassed alleys) was limited out of necessity (to  $n=6$ ) due to the destructive nature of sampling and the difficulty of excavation in the stony soil of our site. However, the fact that the cone biomass and trunk size of the six excavated vines were within the standard error margin of those in the experimental blocks indicates that they were unlikely to have differed strongly in terms of vegetative growth. Furthermore, all cultural management practices were accounted for in biometric measurements conducted in the experimental blocks. These factors are therefore not expected to have contributed greatly to the discrepancy with NPPflux.

There were also approximations implicit in calculating each below-ground term that are worth considering. The NPP determined for the coarse vine roots ( $NPP_{w_{BG}}$ ) is likely an underestimate as it does not include coarse root mortality, and is furthermore an average value for the whole lifespan of the grapevines. Since annual root production in plants may vary according to numerous factors including age (López et al., 2001), this average may differ from the production of 2021. Calculations of fine root production ( $NPP_{fr}$  and  $NPP_{g_{BG}}$ ) were based on turnover rates from literature which might not have accurately represented our site due to pedoclimatic, management and genetic (rootstock, cultivar, and grassed alley species composition) factors. The study by Leifeld et al. (2015), from which we estimated

the grass root turnover rate at our site, was selected because it focused on the fine root turnover rates of montane and alpine permanent grasslands in Europe with similar management practices like regular mowing. However, these sites were located at higher altitudes than ours and likely had different species composition. The resulting NPP values are therefore to be regarded with caution.

Additionally, the yearly flux values should be considered within the meteorological context of that growing season, not only when comparing different vineyard sites but also on a temporal level. While the positive net carbon balance of our vineyard indicates that it was a C sink, this value is based on a single growing season and vineyard NEP has been shown to exhibit high interannual variability (Vendrame et al., 2019). SOC - and to a lesser extent the C stocks of the grapevines - complement the flux values by providing an indication of the vineyard's historical balance. Our results show that the studied site has at the very least been able to maintain a high level of C stocks over the course of its lifespan, primarily through the input of organic matter from the plant residues into the soil. However, the importance of the unirrigated grassed alleys in combination with the deleterious effect of the drought periods on NEP indicate that similar vineyards might become less effective as C sinks in the future as heatwaves and other climate change-related meteorological phenomena become more frequent (Ganguly et al., 2009; Perkins et al., 2012; Russo et al., 2015; Sippel et al., 2018). The new climate characteristics may also redefine the effective storage capacity of the soil, threatening the existing soil C stocks (Moinet et al., 2023), which make up most of the vineyard carbon.

Lastly, the number of variables related to management and site conditions make any inference from a single case study inappropriate. This is especially relevant when assessing the impact of practices like cover cropping on ecosystem C storage, and calls for more case studies in other contexts.

## 5 Conclusion

At our experimental grassed vineyard, we observed a much larger GPP than previously reported for vineyards and many other terrestrial ecosystems, bearing most similarity to managed grasslands.

However, Reco was similar in magnitude and surpassed GPP in the final three months of the season. The agroecosystem therefore acted as a net sink from May to August, with relatively small but positive seasonal value of NEP, of which 33.7% was exported as harvested fruit.

Most of Reco was attributed to Rs (83%), which contained a large net heterotrophic component (Rh: 52% and  $R_{aBG}$ : 48%). Of the total assimilated C that was diverted to plant growth (NPP<sub>biom</sub>), 39.6% was allocated to grapevine plant growth and 60.4% into the growth of the grassed alleys, emphasizing the importance of green manure in short-term vineyard carbon accumulation.

Of the carbon gained during the growing season that remains after harvest (NECB,  $163 \pm 54 \text{ g C m}^{-2}$ ), only 24.2% was attributed to the increase in biomass of permanent grapevine organs. The remainder therefore consisted of an increase in the SOC and undecomposed litter fractions deriving from temporary vegetative structures produced and lost by plants in the same season (fine roots, grass and vine shoots). Most of this C input was lost via decomposition, with only a small fraction contributing to these pools.

SOC was the most important C pool in the agroecosystem, accounting for nearly 13 times more C in the upper 1 m of soil than in all living plant biomass at harvest time. The SOC was comparable with other perennial cropping systems and the lower range of forests, but much smaller than that of grasslands, indicating that GPP was not a strong predictor of soil C in this case.

This study reports a comprehensive carbon budget for a grassed mountain vineyard both in terms of standing C stocks and fluxes during a growing season. Although these results are case-specific, the

detailed insight provided may be used as a reference for future studies aiming to assess vineyard C sequestration in different contexts or explore topics like cover cropping for GHG mitigation in greater depth.

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## Main Figure captions

Fig. 1. Daily carbon fluxes derived from CO<sub>2</sub> flux measurements during the growing season. A) GPP, Reco and NEP derived from eddy covariance data. B-D) Soil respiration fluxes, keeping Reco as a reference: B) Reco and Rs; C) Reco and Rh; D) Reco and Ra<sub>BG</sub>.

Fig. 2. Partitioning of standing carbon stocks at harvest time between different organs in the grapevines and grassed alleys. The grapevine values shown are an average of both cultivars. Errors shown are the SEM of the total carbon represented by each vegetation category.

Fig. 3. Monthly NPP of above-ground grassed alley and vine organs during the growing season as measured using biometric methods. Errors shown are the SEM of the total NPP measured

each month. More detailed information is given in Table 2 (Appendix B – Supplementary Material).

Fig. 4. Depth profile of soil characteristics. A) bulk density; B) Carbon content; C) Nitrogen content.

Fig. 5. Sankey plot showing the fate of carbon within the agroecosystem. Values shown are in  $\text{g C m}^{-2}$  and represent the total fluxes for the growing season (April 15th - November 15th, 2021)  $\pm$  SEM. Values which do not have borders indicating the means of measurement were derived from other totals shown. OF indicates organic fertilizer input.



## Appendix A – Glossary

This is intended as a reference for readers and therefore SHOULD BE INCLUDED IN THE MAIN DOCUMENT.

GPP: gross primary productivity

Reco: ecosystem respiration

NEP: net ecosystem productivity

NBP: net biome productivity (=NECB: net ecosystem carbon balance)

Rs: soil respiration

Rh: heterotrophic respiration

Ra: autotrophic respiration

Ra<sub>BG</sub>: below-ground autotrophic respiration

Ra<sub>AG</sub>: above-ground autotrophic respiration

NPPflux: net primary productivity derived from CO<sub>2</sub> flux measurements

NPPbiom: net primary productivity derived from measurements of plant biomass

NPPc: net primary productivity of canes

NPPl: net primary productivity of leaves

NPPf: net primary productivity of fruits

NPP<sub>fr</sub>: net primary productivity of fine roots

NPP<sub>w<sub>AG</sub></sub>: above-ground woody net primary productivity

NPP<sub>w<sub>BG</sub></sub>: below-ground woody net primary productivity

NPP<sub>g<sub>AG</sub></sub>: above-ground net primary productivity of the grassed alley

NPP<sub>g<sub>BG</sub></sub>: below-ground net primary productivity of the grassed alley

SB<sub>cane/leaf</sub>: standing biomass of the canes or leaves

T<sub>air</sub>: daily average air temperature

VPD: vapor pressure deficit

SWC: soil water content

R<sub>g</sub>: global radiation

DW: dry weight

L<sub>avg</sub>: average length of the shoots

N<sub>s</sub>: number of shoots

OF: organic fertilizers/amendments

SOC: soil organic carbon

## Appendix B – Supplementary Material

This is intended as a supplementary section NOT TO BE INCLUDED IN THE MAIN DOCUMENT. It should contain Figures S1-S5 and Tables S1-S4 along with their captions (see below). These figures are uploaded along with the main text figures.

### Supplementary figure and table captions

Fig. S1. Climatic variables measured at Plantaditsch experimental site during the growing season. A) Rainfall, irrigation and SWC; B) VPD and Tair; C) R<sub>s</sub>.

Fig. S2. Characterization of the eddy covariance measurements in the experimental vineyard. A) Energy balance closure (LE+H vs. R<sub>n</sub>-G) during the growing season (May to October 2021). Latent heat (LE), Sensible heat (H), Net radiation (R<sub>n</sub>) and soil ground flux (G). Points represent half-hourly values. The red line denotes the simple linear regression (equation, R<sup>2</sup> and p-value shown), and the blue line is the ideal 1:1 line. B) Footprint map defined following Kljun et al. (2015).

Fig. S3. Schematic diagram of experimental block layout A) and composition B). In A), vine rows are represented by parallel lines broken into subdivisions by dashed lines. The arrow indicating “slope” points downhill. In B), Rh indicates soil respiration collars on trenched plots and Rs indicates collars on control plots. The green squares show the areas from which respective grass cover samples were taken. Due to the difference in planting density, blocks containing Sauvignon blanc vines were each 5.1 m long and had six vines within (blocks 1-3), whereas those with Chardonnay were 5.25 m long and contained seven vines (blocks 4-6).

Fig. S4. Schematic of the sampling pattern used during the vine excavation (16th of November). A-C refers to the vine replicates and 1-3 the surface position of the soil depth profile for each replicate. Solid red lines indicate how each extracted vine was divided for measurement of DW. The dashed rectangle around vine C represents the zone from which the root system was extracted. X is the inter-vine distance. Figure created using components available under the Creative Commons (<https://creativecommons.org/>) license (Dessi, 2008; OpenClipArt).

Fig. S5. Monthly carbon balance measured by eddy covariance. Values shown by the bars are the mean of results produced using night-time and daytime estimation methods with and without  $u^*$ -selection. The error bars show the standard error between these methods.

Table S1. Carbon budgets of vineyards around the world measured by eddy covariance.

Table S2. List of herbaceous plant species identified at the study site. This list shows only species which were identified by the authors during field work and is not exhaustive. Some spontaneous species in the vine row were also present to a lesser extent in the inter-row and vice versa.

Table S3. Carbon content and other properties of ecosystem carbon pools. For the various grapevine organs, the dry mass (DM) per vine is also shown. The grassed alley carbon pools are totals of the measured seasonal carbon accumulation shown in Table 3.

Table S4. NPP of different ecosystem components for the measured intervals throughout the growing season. All NPP values are in  $\text{g C m}^{-2}$ .

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**Declaration of interests**

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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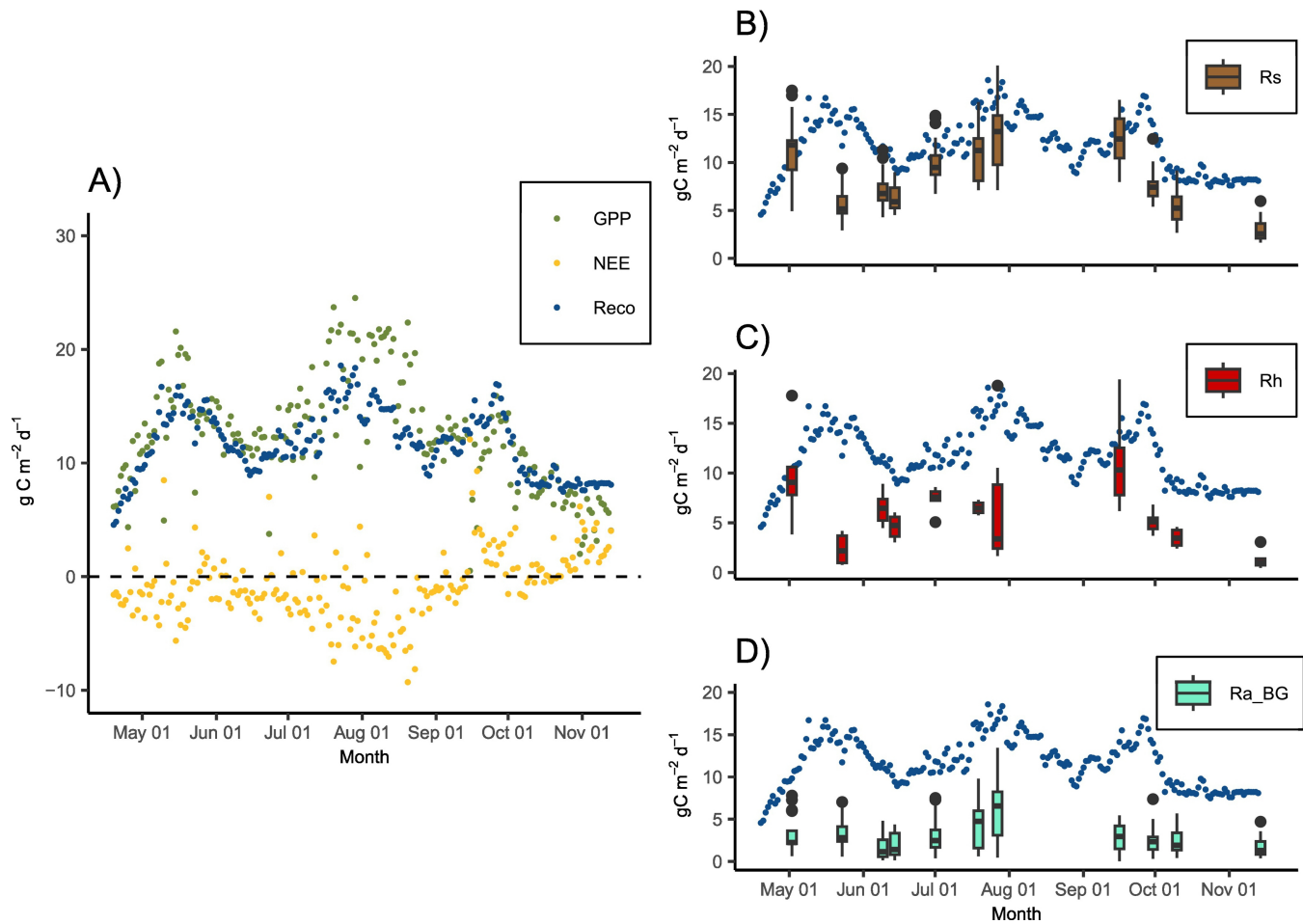


Figure 1

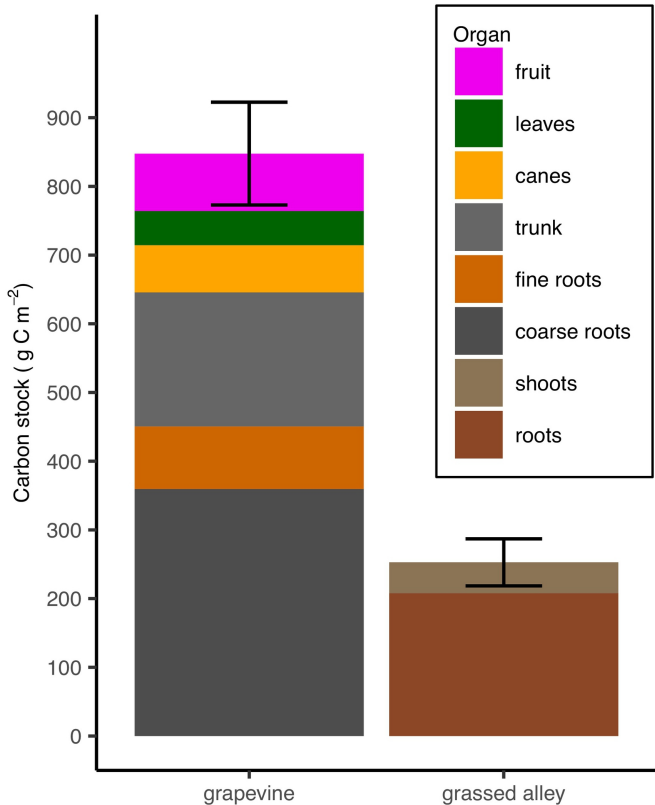


Figure 2



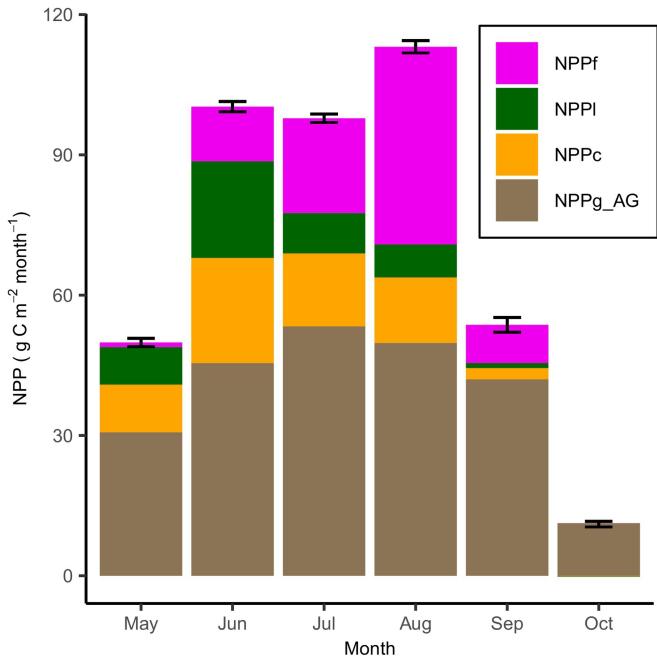
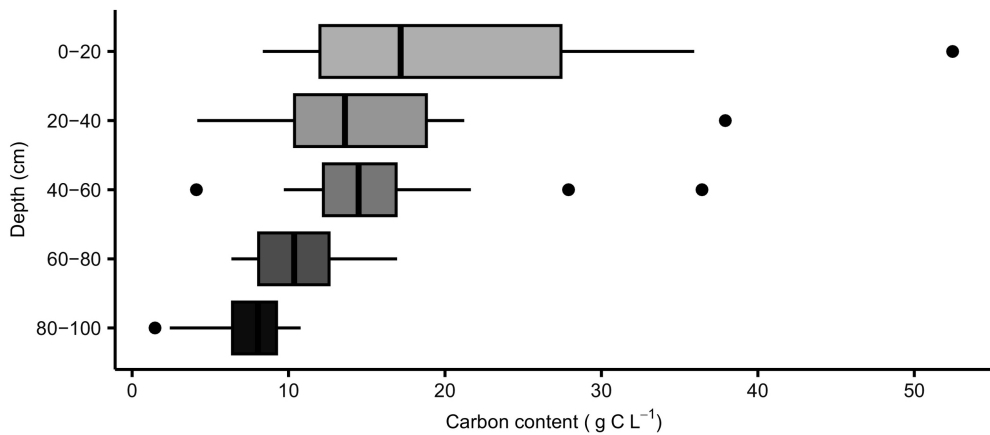


Figure 3

A)



B)

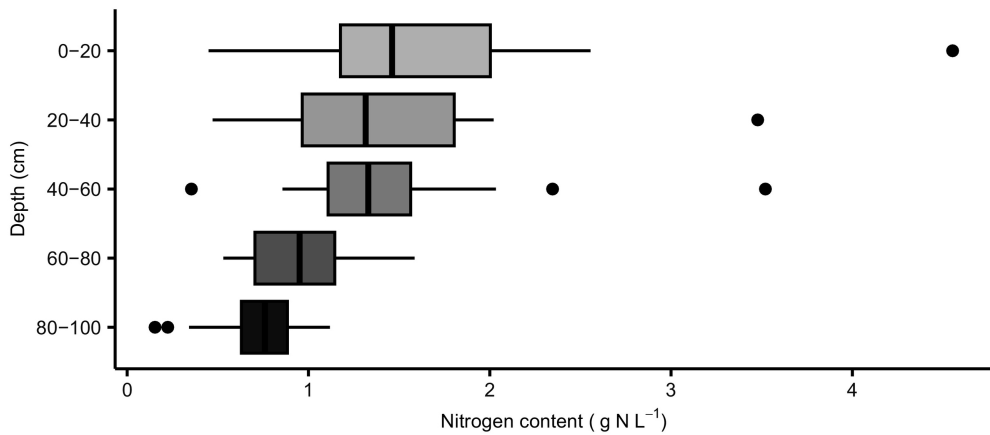


Figure 4

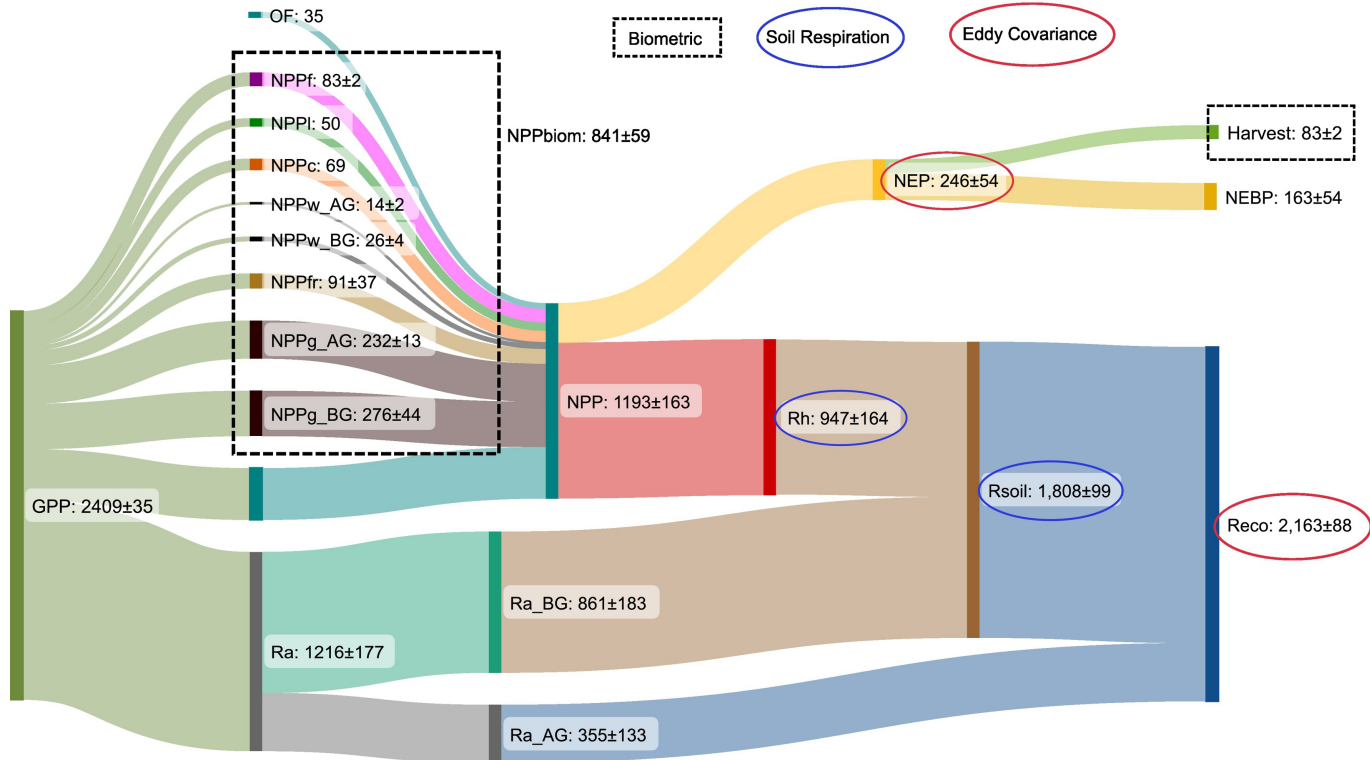


Figure 5