Research Square

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Grazing within Regenerative Perennial Systems Accelerates Soil Carbon and Nitrogen Accrual

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Research Article

Keywords: Integrated crop-livestock systems, agroecology, regenerative agriculture, vineyard, soil organic carbon stocks, soil nitrogen stocks, carbon sequestration, grazing, climate-smart agriculture

Posted Date: April 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9350796/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Global agricultural intensification and the decoupling of crops and livestock systems have reduced soil carbon and nitrogen stocks, weakening terrestrial carbon sinks and altering nutrient cycling. Integrating livestock into cropping systems is a cornerstone regenerative practice, with the potential to enhance ecosystem services, including carbon and nitrogen storage, building blocks of soil health. However, given limited on-farm research quantifying outcomes of stacked regenerative production models, it remains unclear how grazing, alone or in combination with other regenerative practices, affects soil carbon and nitrogen accrual rates. This uncertainty limits the extent to which regenerative models can be supported and scaled. To address this gap, we assessed carbon and nitrogen stocks (0–60 cm), including mineral-associated and particulate soil organic matter pools, across 49 vineyards spanning a gradient of conventional to regenerative management, with and without sheep grazing, in California (USA). We evaluated the relative contributions of regenerative management practices to carbon and nitrogen pools while accounting for spatial and edaphic predictors and estimated accrual rates using space-for-time substitution.

Results

Grazing emerged as the most influential management practice for increasing soil organic matter pool stocks across modeling approaches, followed by reduced physical disturbance. Other regenerative practices, including organic amendments, avoidance of synthetic insecticides, and permanent ground cover, showed positive but more context-dependent associations with soil organic matter pool stocks. Grazing accelerated nitrogen accrual more than five-fold, including plant-available nitrogen, and enhanced soil carbon accrual rates within regenerative management systems, exceeding rates observed in conventional ungrazed systems by more than threefold.

Conclusions

Greater nitrogen availability from grazing may have reduced microbial nitrogen limitation, promoting carbon stabilization. Geography and edaphic properties constrained soil carbon and nitrogen accumulation, with spatially buffered cross-validation highlighting the value of empirically grounded, multi-farm datasets for assessing climate-mitigation potential. These results demonstrate that integrating grazing within regenerative perennial systems can help meet mitigation targets while building nitrogen stocks and reducing reliance on synthetic fertilizers. This underscores the importance of conducting research on commercial operations for developing site-specific strategies and predictive tools for scalable soil carbon accounting and climate-smart agricultural policy.

1. Background

Regenerative agricultural systems, where ecosystem services are harnessed to offset synthetic inputs, are proposed as a means to reduce environmental harm while maintaining crop yields and quality (1, 2). In Mediterranean cropping systems, ecological intensification via livestock grazing may provide a path for adapting to and mitigating climate change through increases in soil carbon (C) and nitrogen (N) stocks (3, 4), while lowering farm carbon footprints through reduced inputs (5–7). The mitigation value of such increases in soil carbon have been highlighted globally through initiatives such as the “4 per 1000” initiative, which proposes that small annual increases (0.4%) in soil organic carbon (SOC) stocks could meaningfully offset anthropogenic greenhouse gas emissions (8).

Regenerative agriculture is guided by principles that minimize soil disturbance, reduce synthetic agrichemical inputs, maintain continuous soil cover, maximize plant diversity, and integrate livestock into cropping systems (9–14). Grazing can facilitate the stacking of regenerative practices by enhancing nutrient cycling, while replacing mowing, herbicide applications, and tillage. Herbicide use and tillage limit nutrient and carbon accrual pathways by reducing biodiversity and disturbing soil and vegetation. Conservation agriculture emphasizes continuous living cover and minimal disturbance. However, it often relies on herbicides and synthetic fertilizers to maintain production (15, 16). Organic systems prohibit synthetic inputs but generally rely on tillage and repeated mowing, while facing nitrogen and weed constraints that can increase costs and reduce yields (17, 18). Regenerative management emphasizes the integration of grazers into cropping systems to reduce trade-offs between productivity and environmental outcomes, and can be implemented in organic and non-organic systems (9).

Grazing in regenerative systems has the potential to be a key practice for increasing cropland SOC because it alters the amount and form of C and N inputs into the soil ecosystem (19–21). Although grazing initially reduces aboveground biomass, optimal grazing can promote above and belowground regrowth, increasing plant production when there is sufficient recovery time between grazing events (22, 23). Grazing stimulates root turnover, increases allocation of photosynthates to root systems that promote regrowth and belowground biomass (24), and redistributes nutrients via manure and urine. Together with increased root exudation, this can enhance microbial biomass and activity (25–29), potentially driving soil C and N accumulation (30–32). Because soil organic matter (SOM) formation is constrained by N availability (33, 34),

grazing-induced increases in total soil nitrogen (TSN) and plant-available N has the potential to alleviate stoichiometric limits and promote stabilization of carbon in mineral-associated organic matter, the most stable fraction, while enhancing plant productivity (27). However, the extent to which these mechanisms translate into sustained C and N gains remains highly context dependent and poorly quantified in Mediterranean climate perennial systems, where interacting management practices, water limitation, and the scarcity of long-term studies limit robust inference (29, 35).

Soil organic matter (SOM) accrual is influenced by biotic inputs and abiotic conditions. Finer-textured soils with higher exchangeable calcium (pH > 6.5) are associated with greater SOM due to microaggregate protection, organo-mineral associations, and cation bridging (36–39). Climate further regulates these processes. Gradients in latitude, elevation, and distance from the coast influence rainfall and temperature, with cooler, wetter northern, higher-elevation, and coastal regions generally supporting greater biomass inputs and slower decomposition than warmer, more water-limited environments (40, 41). Because geographic position integrates variation in climate, soils, and landscape context, multi-farm approaches that capture cumulative time under management are needed to quantify how management interacts with environmental constraints to shape soil carbon and nitrogen accrual on commercial operations.

There is a critical need for on-farm research that quantifies soil carbon and nitrogen outcomes of stacked regenerative production models (42). Currently, carbon accounting relies on process-based models validated with small, highly controlled plot studies that seldom reflect farming complexities. This limits their ability to capture practice-driven changes in carbon and nitrogen cycling, constraining inference and confidence in modeled outcomes (43). These studies often capture a narrow subset of management practices and soil-climate conditions relative to the broader range over which models are applied (44) and lack on-farm validations (45) that consider more complex implementation of practices across time and space than is possible in controlled research trials (46). Consequently, process-based models may not accurately predict soil-carbon accrual and their rates at operational scales. Externally validated, multi-farm datasets are therefore essential to improve these models and the monitoring, reporting, and verification systems used to quantify potential soil carbon sequestration and scale soil-carbon accounting (42, 43). Since site-specific temporal SOC data are rarely available for commercial farms, space-for-time substitution across farms with contrasting management histories (47–49) can provide context-rich empirical data to inform carbon accounting frameworks and climate mitigation policies.

California's diverse animal and perennial crop systems provide a strategic landscape for large-scale crop–livestock reintegration to help meet regional climate-mitigation targets. This approach can also strengthen agricultural resilience as drought and warming reduce rangeland forage and grazing mitigates wildfire risk (50). However, the potential of grazing to build soil carbon and nitrogen pools when integrated into perennial cropping systems such as vineyards, and the synergies and trade-offs among co-management practices, remain understudied and poorly understood (35). Quantifying how grazing contributes to soil organic carbon accrual over time, and how it interacts with co-management practices can guide adoption and shape policies that support transitions to regenerative systems.

To address these knowledge gaps, we evaluated how integrating sheep grazing with regenerative management practices influenced soil carbon and nitrogen stocks and accrual in commercial vineyards. To address our first research question (**RQ1**), we assessed whether vineyards with established grazing (≥ 3 years) had higher stocks of plant-available nitrogen (PAN) and six soil organic matter pools: soil organic carbon (SOC), mineral-associated organic carbon (MAOC), particulate organic carbon (POC), total soil nitrogen (TSN), mineral-associated nitrogen (MAN), and particulate nitrogen (PN). Next, because sheep grazing does not occur in isolation, we evaluated the relative importance of grazing, co-occurring management practices, and spatial and edaphic variables on the six SOM stocks (**RQ2**). Finally, we used space-for-time substitution to estimate annual accrual rates and evaluated how grazing interacted with conventional and regenerative management trajectories to influence soil organic carbon, total soil nitrogen, and plant-available nitrogen accrual (**RQ3**). We then tested the predictive capacity of these management histories across the landscape. We hypothesized that livestock integration would function as a keystone practice for soil carbon and nitrogen stocks across a conventional-to-regenerative management gradient. We further hypothesized that grazing would amplify accrual rates when embedded within regenerative management systems, resulting in the highest SOC, TSN, and PAN accrual in regenerative grazed systems. Finally, we hypothesized that the magnitude and rate of soil organic matter accrual would be influenced by geographic and edaphic properties.

2. Methods

2.1 Site selection and design

Between 2022 and 2024, 49 commercial wine grape vineyards distributed across three California regions and seven counties were sampled: North Coast (Mendocino, Lake, Sonoma, Napa), Central Coast (San Benito), and Central Valley (Solano, San Joaquin) (Fig. 1). These regions share a Mediterranean climate (51): hot, dry summers and mild winters. Local microclimates vary according to coastal proximity, latitude, elevation, and surrounding topography. Vineyards were well-established (mean age at sampling ± 95% CI: 17 ± 3.7 years), and livestock integration sites had at least three consecutive years of sheep grazing prior to sampling. Vineyards were paired (grazed, n = 26; ungrazed, n =

23) within 20 km to approximate comparable soil and climatic conditions. Pairs were matched on soil texture, Ca^{2+} saturation, soil pH, grape variety, rootstock, trellising system, vine age, and position along the conventional-to-regenerative management gradient; all sites also practiced winter cover cropping. (Fig. 1; Supplemental Table 1). In 2022, one ungrazed site was determined prior to sampling to have had historical grazing but was retained; in 2024, one grazed Napa vineyard lacked a comparable ungrazed site. Pairing was used for site selection rather than statistical blocking, and statistical analyses treated each vineyard independently. This design minimized differences in soil and management conditions between grazed and ungrazed vineyards, strengthening inference regarding livestock integration effects within this observational framework.

2.2 Vineyard management characterization

Grower surveys were used to document input use and years under different management practices (Supplemental Materials). Each vineyard was characterized along a conventional-to-regenerative gradient using a regenerative management scoring matrix (9). Vineyards received a score of 1 for each regenerative practice adopted or conventional practice avoided for at least 3 years, with a maximum possible regenerative score of 8 (Table 1, Fig. 1). Practices classified as regenerative included: use of organic amendments (52), no-till management (53), prescribed grazing (54), and maintaining winter ground cover via cover cropping (55) or resident vegetation (52, 56, 57). Each of these practices contributed a score of 1 within the binary system. Use of synthetic fertilizers, herbicides, insecticides, or fungicides and the use of tillage were scored as zero. Vineyards with scores ≥ 4 were classified as regenerative, while those scoring < 4 were categorized as conventional. Seventy-one percent (17 of 24) of the established regenerative sites were certified USDA organic. For models assessing management duration, the management states were 1/ conventional ungrazed, 2/ conventional grazed, 3/ regenerative ungrazed, and 4/ regenerative grazed (Supplemental Table 2, Supplemental Fig. 1).

2.3 Soil sampling and processing

Soil sampling was conducted once per vineyard at budbreak (late March to early April). Sixteen vineyards were sampled in 2022, seventeen in 2023, and nineteen in 2024. Three vineyards sampled in both 2022 and 2023 were averaged, yielding a final sample size of 49 vineyards. Four 50 m transects were established along the vine rows at least 20 m apart. For the six SOM pools, from each transect, four soil cores to 60 cm depth were collected at 0 m (aisle), 15 m (margin), 30 m (row), and 45 m (aisle) (Supplemental Fig. 2) using a 2.54 × 91.44 cm Plated Replaceable Tip Probe-Hammer Head Handle (AMS, American Falls, ID). Cores were sectioned into 0–5, 5–10, 10–15, 15–30, and 30–60 cm layers and composited for each layer at the transect level. For plant-available nitrogen (PAN), twelve separate 0–15 cm cores were taken at 4 m intervals along each transect in an aisle–margin–row pattern. Samples were composited at the transect level, refrigerated at 4° C, and sent within 7 days of collection to Regen Ag Labs for analysis (Nebraska, USA). Samples were tested for total soil carbon (TSC), soil organic carbon (SOC), total soil nitrogen (TSN), PAN, Ca^{2+} saturation, exchangeable Ca^{2+}, and soil texture. Samples were assessed for C and N in the mineral-associated and particulate organic matter pools at UC Davis.

2.4 Ground Cover Assessment

Total ground cover (green cover + residue) was estimated visually, twice for each vineyard: at budbreak and at 50% veraison. A 40.64 cm × 40.64 cm quadrant was placed along each 50 m transect, at the 0 m (row), 25 m (margin), and 50 m (aisle) points (supplementary Fig. 2). Values were averaged at the farm level, to derive the binary permanent ground cover variable (> 2/3 cover at both sampling times).

2.5 Soil C and N pools

TSC, SOC, and TSN were measured via dry combustion (LECO CN 932, St. Joseph, MI) of air dried and ground soil samples (2-mm sieved). For SOC, samples were pretreated with Sulfurous Acid to remove inorganic carbon (58). MAOC and POC, along with their nitrogen fractions (MAN, PN), were measured using dry combustion (Costech 4010, Valencia CA) and determined for the 0–15, 15–30, and 30–60 cm depths using aggregate dispersion, wet sieving, and particle-size fractionation (27, 59). The mineral fraction was defined as < 0.053 mm and the particulate fraction as ≥ 0.053 mm. A mass recovery threshold of 95–105% was enforced, and samples outside this range were re-run. Across all soils, the mean recovery was 99.5% with a standard deviation of 1.0%.

Exchangeable and total Ca^{2+} saturation were determined via the ammonium acetate method (60). Soil texture (sand, silt, and clay percentages) was determined using the hydrometer method (61). PAN was measured as the sum of inorganic N (NH_4^+ and NO_3^-) and potentially mineralizable N following the USDA Soil Health Tool protocol (62).

2.6 SOM baseline, stock, and accrual

Carbon and nitrogen accrual stocks were calculated as measured stock minus an internally derived baseline using the Franzluebbers depth-distribution method (63–65). Consistent with the Franzluebbers depth-distribution method (64) accrual within 0–30 cm was defined as **root-zone accrual stocks (RZA)**. This approach is ideal for observational space-for-time studies where pre-treatment baselines are not available.

Baseline concentrations (g kg^{-1}) for SOC, MAOC, POC, TSN, MAN, and PN were estimated using a nonlinear exponential depth-distribution model fit to measured concentrations across 0–15, 15–30, and 30–60 cm depth increments. The predicted concentration at 30 cm was used as the baseline reference for the 0–30 cm interval. The predicted concentration at 60 cm was used as the baseline reference for the 30–60 cm interval. Predicted concentrations were interpolated in 5-cm increments to 60 cm. Actual stock for each depth interval was calculated by summing modeled 5-cm increments multiplied by corresponding bulk density. Baseline stock for each interval was calculated by projecting the respective baseline concentration uniformly across that interval and multiplying by depth-specific bulk density. Bulk density was estimated from SOC using the pedotransfer function of (64, 66) to support standardized stock calculations across sites. Accrual stock was defined as: *Accrual stock = Measured stock − Baseline stock*.

Profiles with nonlinear $R^2 \geq 0.90$ were accepted. Profiles with $0.70 \leq R^2 < 0.90$ or flagged B-parameter outliers were re-fit using a generalized additive model (GAM) and retained only if the GAM achieved $R^2 \geq 0.90$ and $RMSE \leq 1.10\times RMSE_{(NL)}$. Profiles with nonlinear $R^2 < 0.70$ or failing GAM criteria were designated NA.

2.7 Statistical analyses

Data analyses were conducted in R (67) using RStudio (68). Statistical analyses relied on core packages including mgcv (69), gam (70), randomForest (71), vegan (72), lme4 (73), and the tidyverse (74); a complete list of packages used is provided in the Supplemental References. We used a combination of machine learning (Random Forest [RF]) (75), generalized additive modeling (GAM) (76), and multivariate statistical approaches (PERMANOVA) (77) to evaluate the effects of vineyard management on soil carbon and nitrogen accrual (Supplemental Fig. 3). The code and associated data used for these analyses can be found on the USDA Ag Data Commons.

GAM and RF models capture nonlinear ecological patterns and robustly handle nonparametric data. GAMs were fit with maximum likelihood (ML) for dredging for AICc comparability across models. A restricted maximum likelihood (REML) was used for individual model estimation. GAMs used thin-plate regression splines with basis size set by predictor type, larger for the bivariate spatial surface (longitude, latitude; k = 25) and smaller for univariate edaphic terms (clay %, silt %, Ca^{2+} saturation, exchangeable Ca^{2+}; k = 7). Basis adequacy was checked with gam.check. Management predictors were treated as linear parametric terms. To mitigate overfitting and promote parsimony, dredge candidate models used thin-plate regression splines (bs = 'tp') for the bivariate spatial surface and thin-plate shrinkage smooths (bs = 'ts') for edaphic terms, allowing weak edaphic smooths to shrink toward zero while preserving a consistent model structure for AICc comparability. Individual model fits used thin-plate regression splines (bs = 'tp') throughout, with whole-term penalization (select = TRUE) to allow complete smooth shrinkage. Exchangeable Ca^{2+} was included in dredge models but dropped from individual models, as Ca^{2+} saturation better reflects calcium availability relative to other cations. The basic structure of the GAMs is shown below.

Response ~ Treatment + s(Longitude, Latitude) + s(Silt) + s(Clay) + s(Ca^{2+} saturation)

2.7.1. Importance of management, spatial, and edaphic factors on accrued stocks across the six SOM pools (RQ1 & RQ2)

To address RQ1 GAMs (REML) were used to evaluate binary contrasts for grazing (yes/no) in each of the six RZA SOM pools and PAN (Supplemental Table 3). For RQ2, RF and GAM approaches were combined to assess the relative importance of spatial and edaphic variables and management practices (Table 1) across the six RZA (0–30 cm) SOM pools. RF models (randomForest; ntree = 1000, averaged across 10 runs) provided rankings of variable importance (Supplemental Table 4), while GAMs (mgcv; ML) estimated effect direction and significance. To better quantify the relative importance of management practices in statistical analyses, individual practices were refined (Table 1). Tillage was partitioned into no-till tractor row and no-till under-vine components to reflect distinct spatial niches, while OMRI-certified NPK fertilizers were included within organic amendments. Because winter cover cropping was universally adopted across sites, permanent ground cover (>□ at budbreak and ~ 50% veraison) was used instead as a variable in the management-practice importance assessment (Table 1). GAM selection was performed using MuMIn::dredge, retaining all models within ΔAICc ≤ 7 (78). Predictor importance was summarized using Weighted Scores calculated as the summed contribution of each predictor across supported models. For each predictor **j**, we summed its contribution across all top models **i**:

$$WeightedScore_j = \sum\nolimits_i w_i I\left(p_{ij} < 0.05\right)$$

where $\mathbf{w_i}$ is the Akaike weight of model **i** (re-normalized to sum to 1 within the ΔAICc ≤ 7 model set), and $\mathbf{I(p_{ij}}<0.05)$ is an indicator equal to 1 if predictor **j** was statistically significant in model **i** and 0 otherwise.

To assess the relative influence and directional effects of vineyard management practices on the soil RZA C and N pools, we integrated RF importance with unconditional GAM inference across the full candidate model set (Supplemental methods). RF models quantified predictor importance based on predictive performance. GAMs provided effect direction and uncertainty using unweighted meta-analytic estimates derived from model-averaged coefficients and their within- and between-model variance.

To evaluate management effects across RZA pools, RF-derived importance ranks were combined with GAM-based Weighted Scores and averaged across RZA SOM fractions. Directional markers indicate predictors that showed convergent effects across multiple SOM pools, capturing both the magnitude and stability of management while accounting for model uncertainty (Fig. 3).

For the six individual SOM pools directional inference was based on sign dominance criteria that distinguished statistically supported effects ($p < 0.05$), marginal effects (0.05–0.10), and direction-only associations, allowing consistent but weak effects to be retained where directional agreement was high. These metrics were synthesized into pool-specific RF–GAM summaries for the six RZA SOM fractions (Fig. 4).

2.7.2. Accrual rates and management-duration effects (RQ3)

Multivariate differences in the six RZA SOM pools and plant-available N accrual rates were assessed using PERMANOVA with Bray–Curtis dissimilarities and 999 permutations (adonis2, vegan) to evaluate how management history and environmental gradients structured multivariate accrual patterns. Continuous covariates (longitude, latitude, silt, clay, and Ca^{2+} saturation) were standardized prior to analysis. The model included years under each management trajectory (conventional ungrazed, conventional grazed, regenerative ungrazed, regenerative grazed), spatial coordinates, and soil covariates. Marginal tests (by = "margin") were used to obtain order-invariant partial R^2 and F statistics for each predictor while holding all other variables constant.

Accrual rates of SOC (RZA), TSN (RZA), and PAN were modeled using GAM (REML) with spatial and soil covariates as thin-plate regression spline smooths. Management history was represented as continuous years under conventional ungrazed (Cu), conventional grazed (Cg), regenerative ungrazed (Ru), and regenerative grazed (Rg) management (Supplemental Table 2, Supplemental Fig. 1). Total years across these categories were summed and centered, such that the centered term provided the per-year slope for conventional ungrazed (Cu) management, whereas the deviation terms (d_Cg, d_Ru, d_Rg) captured per-year differences for conventional grazed (d_Cg), regenerative ungrazed (d_Ru), and regenerative grazed (d_Rg) systems relative to conventional ungrazed management. Per-year accrual trajectories and contrasts among management systems were obtained using linear contrasts of the re-parameterized GAM coefficients. Statistical significance was evaluated using two-sided t-tests based on each contrast estimate, its standard error, and the model's residual degrees of freedom.

To evaluate whether environmental context modified management-driven accrual trajectories, interaction GAMs were fitted. Management-specific slopes were allowed to vary across spatial and edaphic gradients. Specifically, tensor-product smooths with "by" variables allowed slopes for the centered conventional ungrazed trajectory (cT) and deviation terms (d_Cg, d_Ru, d_Rg) to vary across geographic space (longitude–latitude) and soil properties (Ca^{2+} saturation, silt, and clay). Models were fitted using ML to compare with the baseline GAM, and interaction support for SOC, TSN, and PAN accrual was evaluated using likelihood ratio tests, AIC, adjusted R^2, and interaction p-values (Supplemental Table 5). Spatial prediction maps were generated from fitted GAMs by varying longitude and latitude across the observed vineyard extent while holding non-spatial covariates at their median values and management terms at zero (Fig. 6). Predictions were masked to areas near sampled vineyards to avoid unsupported extrapolation. County boundaries and vineyard locations were overlaid for geographic reference. Partial effect plots for Ca^{2+} saturation, silt, and clay were extracted from the fitted GAM smooths with 95% confidence intervals.

Predictive capacity (79, 80) of management duration for RZA SOC and TSN was evaluated using five-fold spatially blocked cross-validation (CV) (81), with coordinates projected to UTM Zone 10N and block size set to 1.5× the spatial autocorrelation range (82). Each five-fold spatial CV was repeated 20 times, with models trained on four folds and validated on the fifth. With each repetition, data points were redistributed into new spatial blocks, generating a new five-fold split. Performance was then pooled across folds and repetitions to obtain RMSE and associated 95% confidence intervals.

Management practices of the sampled California vineyards

Table 1
Management practices of the sampled California vineyards. Mean implementation percentages (± 95% CI) are shown for each binary management practice, summarized across vineyards classified by sheep grazing (grazed [≥ 3 years] vs. ungrazed). The ± 95% confidence intervals reflect vineyard variability within each management group and represent the uncertainty in the estimated group mean implementation percentage. Vineyards received a score of 1 for each regenerative practice adopted or conventional practice avoided, yielding a maximum possible regenerative score of 8 (Fenster, LaCanne et al., 2021). Practices earning a score of 1 included the use of organic amendments, no-till, winter cover crops, and the integration of sheep grazers. Use of synthetic fertilizers, herbicides, insecticides, and fungicides received a score of 0, whereas avoiding these inputs received a score of 1. Vineyards with scores ≥ 4 were classified as regenerative and those with scores < 4 as conventional. To better quantify management practice importance, tillage was divided into no-till tractor row and no-till under vine to reflect spatial niches, while OMRI-certified NPK fertilizers (e.g., poultry litter, compost tea) were added to organic amendments. Because winter cover cropping was universally adopted, permanent ground cover (>□ at budbreak & at ~ 50% veraison) was used to assess management practice importance. See Fig. 1 for the geographic distribution of the sites as well as the distribution of conventional-regenerative scores among grazed and non-grazed vineyards.

Grazing Practice	n	No Synthetic Fertilizers	No Synthetic Herbicides	No Synthetic Fungicides	No Synthetic Insecticides	Organic Amendments-Fertilizers	No-Till Tractor Row	No-Till Undervine	Winter Cover Crops	Permanent Ground Cover
Grazing	26	65 ± 20	85 ± 15	65 ± 20	77 ± 17	73 ± 18	58 ± 20	42 ± 20	100 ± 0	42 ± 20
No Grazing	23	26 ± 19	35 ± 21	26 ± 19	39 ± 22	78 ± 18	30 ± 20	65 ± 21	100 ± 0	4 ± 9

3. Results

3.1 Soil carbon and nitrogen accrual is concentrated in the upper 30 cm

Accrued stocks of soil carbon and nitrogen pools (measured stocks – estimated baseline stocks) were concentrated in the top 30 cm of soil, particularly within the 0–15 cm layer (Table 2; Fig. 2). This pattern was consistent across treatments and soil organic matter (SOM) pools. Although the 30–60 cm depth showed no treatment differences, both the grazed and ungrazed treatments accrued carbon and nitrogen at these depths (Fig. 2). Consequently, analyses examining relationships between management and SOM pool accrual focused on the top 30 cm, defined as Root Zone Accrual stocks (RZA) (5, 63, 64, 83). Estimated baseline, observed, and accrued stock quantities for each treatment category are in Supplemental Fig. 4.

3.2 Established grazing increases soil carbon and nitrogen RZA stocks across pools

For the following results, the mean and CIs (SD / √n) are the untransformed values. The p-value is the number associated with the established grazing management coefficient in the GAM model. The R_{adj}^2 values reflect models that included spatial and edaphic smooth terms alongside the grazing predictor; full model details including smooth term significance and effective degrees of freedom are provided in Supplemental Table 3. Sites with established grazing regimes (≥ 3 years) had significantly higher RZA stocks across C pools compared to non-grazed sites (Fig. 2A). Vineyards that integrated grazing stored 21.50 ± 2.98 Mg soil organic carbon (SOC) ha^{-1}, compared to 16.47 ± 3.54 Mg SOC ha^{-1} without grazing, a 31% increase (N = 49, P < 0.001, R_{adj}^2 = 0.78). Mineral-associated organic carbon (MAOC) was 27% higher in grazed vineyards which stored 12.28 ± 2.18 Mg ha^{-1} compared to 9.68 ± 2.26 Mg ha^{-1} in ungrazed vineyards (N = 47, P = 0.034, R_{adj}^2 = 0.73). Particulate organic carbon (POC) was 43% higher under grazing (9.99 ± 1.50 Mg ha^{-1} compared to 6.96 ± 1.93 Mg ha^{-1} in non-grazed vineyards; N = 49, P = 0.002, R_{adj}^2 = 0.47).

Similarly, grazed vineyards had larger N pools (Fig. 2B). Total soil N (TSN) was 38% higher in soils with grazing (1.68 ± 0.22 Mg ha^{-1}) versus soils without grazing (1.22 ± 0.21 Mg ha^{-1}) (N = 49, P < 0.001, R_{adj}^2 = 0.81). Mineral associated nitrogen (MAN) increased by 47%, averaging 1.03 ± 0.19 Mg ha^{-1} under grazing compared with 0.73 ± 0.12 Mg ha^{-1} in non-grazed vineyards (N = 47, P < 0.001, R_{adj}^2 = 0.67). Particulate nitrogen (PN) was 77% higher under grazing (0.84 ± 0.24 vs. 0.47 ± 0.14 Mg ha^{-1}; N = 47, P = 0.013, R_{adj}^2 = 0.21). Finally, plant-available nitrogen (PAN) was 21% higher in grazed vineyards, (32.51 ± 4.46 vs. 26.82 ± 3.65 kg ha^{-1}, N = 49, P = 0.032, R_{adj}^2 = 0.27).

Depth-wise distribution of the accrued C and N stocks (measured – estimated baseline) across SOM pools in California wine grape vineyards

Table 2
Depth-wise distribution of the accrued C and N stocks (measured – estimated baseline) across SOM pools in California wine grape vineyards. For each SOM pool, % share of the pool's aggregated mass in each depth increment (0–15, 15–30, 30–60 cm) is shown. Soil organic carbon (SOC), mineral-associated organic carbon (MAOC), particulate organic carbon (POC), total soil nitrogen (TSN), mineral-associated nitrogen (MAN), particulate nitrogen (PN). The final column reports the average pool mass per vineyard over 0–60 cm (Mg ha^{-1}). Percentages rounded to 0.1% using a largest-remainder method to ensure they sum to 100.0% across depths.

SOM Pool	0-15cm	15-30cm	30-60cm	Average Mass (Mg/ha)
SOC	70.0%	14.3%	15.7%	22.7
MAOC	62.8%	14.9%	22.3%	14.2
POC	83.0%	10.7%	6.3%	9.1
TSN	69.8%	14.3%	15.9%	1.7
MAN	65.5%	14.4%	20.1%	1.1
PN	89.7%	6.0%	4.3%	0.7

3.3 Spatial and edaphic gradients, alongside management, shape soil carbon and nitrogen RZA stocks across vineyards (RQ2)

3.3.1 Averaged across the six soil C and N pools, spatial and edaphic predictors are strongest, with grazing as the most consistent management driver

To evaluate management effects across RZA pools, predictor importance was summarized by averaging Random Forest importance ranks (lower rank = greater importance) and GAM-derived Weighted Scores (higher score = greater importance) across the six RZA SOM fractions. Spatial variables were the strongest predictors across the six RZA SOM pools, followed by silt and clay content (Fig. 3). Regarding management practices, in the RF analysis, the leading management predictors were avoiding synthetic insecticides (7.0 ± 2.4), grazing (7.2 ± 3.6), and avoiding synthetic fertilizers (8.0 ± 2.8) (Fig. 3). GAMs identified maintaining permanent ground cover (0.55 ± 0.06), no-till in the tractor row (0.36 ± 0.07), and integrating livestock (0.34 ± 0.07) as practices with the highest weighted scores when averaged across SOM pools (Fig. 3). Grazing was the only management practice that ranked within the top 3 variables for both modeling approaches.

3.3.2 Spatial and edaphic gradients structure soil carbon and nitrogen pools, with pool-specific management effects

Predictors of RZA carbon (SOC, MAOC, POC) and nitrogen (TSN, MAN, PN) pools were evaluated using Random Forest (RF) models to assess variable importance and generalized additive models (GAMs) to estimate marginal relationships. Across pools, RF models explained substantial variance (R^2 = 0.90–0.96), with spatial (latitude, longitude) and edaphic variables consistently ranking highest.

Soil Organic Carbon (SOC)

SOC RZA stocks were strongly structured by spatial–edaphic gradients (Supplemental Table 4). No-till in the tractor row provided the clearest management signal, with a high RF ranking while also emerging as a significant positive predictor in the GAMs (β = 5.00, p = 0.008, WS = 0.73) (Fig. 4A). In contrast to RF models, GAMs identified permanent ground cover as a significant positive predictor of SOC (β = 5.24, p = 0.009, WS = 0.92). Variation in SOC RZA stocks was well explained by RF (R^2 = 0.96), and retained GAMs showed strong explanatory power (mean R^2 = 0.725 ± 0.004).

Mineral-Associated Organic Carbon (MAOC)

Longitude and latitude were the strongest predictors of MAOC RZA stocks (Supplemental Table 4). Among management variables, organic inputs and no-till in the tractor row ranked relatively high in RF importance (Fig. 4B). In the GAMs, permanent ground cover was a significant positive predictor of MAOC (β = 2.87, p = 0.019, WS = 0.79) despite negligible RF importance. Most of the variation in MAOC was explained by the RF analysis (R^2 = 0.93), with GAMs also demonstrating strong explanatory power (mean R^2 = 0.757 ± 0.005).

Particulate Organic Carbon (POC)

While latitude and longitude again explained most of the variation (Supplemental Table 4), management variables exhibited greater influence relative to the other carbon pools within the RF analysis. Reduced synthetic herbicide use, integrating livestock, and reduced insecticide use

ranked highly (Fig. 4C), while GAMs identified permanent ground cover (β = 3.40, p = 0.02, WS = 0.83) as a significant positive predictor. RF explained substantial variance in POC (R^2 = 0.903), whereas GAM explanatory power was lower (mean R^2 = 0.467 ± 0.001).

Total Soil Nitrogen (TSN)

Longitude, total exchangeable Ca^{2+}, and clay content were the leading RF predictors (Supplemental Table 4). Regarding management, both modeling approaches identified grazing (β = 0.35, p = 0.002, WS = 0.991) as a key driver of TSN RZA stocks (Fig. 4D). No-till in the tractor row (β = 0.24, p = 0.040, WS = 0.45) and permanent ground cover (β = 0.30, p = 0.041, WS = 0.42) were also significant positive predictors, despite more modest RF rankings. RF explained nearly all variation in TSN (R^2 = 0.95), and GAMs showed the highest explanatory power among pools (mean R^2 = 0.778 ± 0.005).

Mineral-Associated Nitrogen (MAN)

Longitude, total exchangeable Ca^{2+}, and clay content were the strongest RF predictors for MAN (Supplemental Table 4). Among management variables, grazing ranked highest in RF (Fig. 4E). Although grazing showed a marginally significant positive effect on MAN (β = 0.18, p = 0.071), its weighted score (WS = 0.44) was substantially lower than for TSN (WS = 0.991), reflecting greater model dependence and cross-model variability in the MAN response (Fig. 4E). No-till in the tractor row was also identified as a significant positive predictor in GAMs (β = 0.26, p = 0.005, WS = 0.96), although its RF importance was more limited (Fig. 4E). RF explained substantial variation in MAN (R^2 = 0.94), with GAMs showing moderate explanatory power (mean R^2 = 0.587 ± 0.006).

Particulate Nitrogen (PN)

For PN, latitude was the strongest predictor, followed by longitude, avoiding synthetic insecticides, and integrating livestock (Supplemental Table 4). Among management variables, avoiding synthetic insecticides ranked highest, with the GAMs indicating a positive direction-only association (β = 0.35, p = 0.226, WS = 0.050). Grazing ranked second in RF importance and was the only significant predictor identified by the GAM analysis (β = 0.32, p = 0.044, WS = 0.54) (Fig. 4F). The RF analysis explained most of the variation in PN (R^2 = 0.91), whereas GAM explanatory power was comparatively low (mean R^2 = 0.176 ± 0.005).

3.4 Regenerative and grazed management histories increase soil C and N accrual (RQ3)

3.4.1 Multivariate soil C and N accrual is driven by years under regenerative management

Accrual in the six RZA SOM pools and plant-available N (PAN) varied significantly with years under regenerative management. PERMANOVA indicated significant associations between accrual patterns and years under regenerative management in both grazed (R^2 = 0.063, p = 0.008) and ungrazed systems (R^2 = 0.057, p = 0.014), whereas years under conventional ungrazed (R^2 = 0.032, p = 0.063) and conventional grazed management (R^2 = 0.028, p = 0.088) were not significantly associated with multivariate variation in accrual. Spatial variables were also strong predictors, with significant effects of longitude (R^2 = 0.140, p = 0.001) and latitude (R^2 = 0.070, p = 0.005). Soil texture and Ca^{2+} saturation showed no detectable relationships (silt R^2 = 0.005, p = 0.71; clay R^2 = 0.008, p = 0.53; Ca^{2+} saturation R^2 = 0.023, p = 0.16).

Across the six SOM pools and PAN, management, spatial, and soil predictors together explained ~ 43% of the variation, with ~ 45% attributed to residual variation. To quantify directional changes in individual pools, we next modeled accrual trajectories using generalized additive models (GAMs).

3.4.2 Management history drives accrual in individual soil C and N pools

Soil Organic Carbon (SOC)

Management trajectory significantly influenced RZA SOC accrual after adjusting for spatial and soil covariates (R^2_{adj} = 0.80) (Table 3, Fig. 5). Annual SOC gains were positive and significant in conventional ungrazed vineyards (0.17 ± 0.08 Mg SOC ha^{-1} yr^{-1}, p = 0.042) and higher under regenerative management (0.34 ± 0.09 Mg SOC ha^{-1} yr^{-1} for ungrazed, p < 0.001; 0.55 ± 0.15 Mg SOC ha^{-1} yr^{-1} for grazed, p < 0.001). Conventional grazed vineyards had a marginally significant absolute slope (0.59 ± 0.32 Mg SOC ha^{-1} yr^{-1}, p = 0.070).

Relative to the centered conventional–ungrazed accrual rate, regenerative grazed vineyards accrued significantly more SOC (+ 0.38 ± 0.13 Mg SOC ha^{-1} yr^{-1}, p = 0.006) (Table 3, Fig. 5), while regenerative–ungrazed vineyards showed a smaller, marginal increase (+ 0.17 ± 0.09 Mg SOC ha^{-1} yr^{-1}, p = 0.066) (Table 3). Conventional grazed vineyards did not differ significantly from the baseline (+ 0.42 ± 0.31 Mg SOC ha^{-1} yr^{-1}, p = 0.192), with regenerative and conventional grazed vineyards not differing within the grazed sites (–0.04 ± 0.32 Mg SOC ha^{-1} yr^{-1}, p = 0.902) (Table 3).

Spatial effects were dominated by the 2-D longitude–latitude smooth ($p < 0.001$), with Ca^{2+} saturation also exerting a significant nonlinear influence ($p < 0.001$). Smooths for silt and clay were non-significant ($p = 0.174$ and $p = 0.134$). Overall, regenerative management amplified SOC gains, with the largest and only significant increase relative to the conventional ungrazed accrual rate observed when grazing was integrated within regenerative systems.

Total Soil Nitrogen (TSN)

Grazing strongly influenced RZA TSN accrual when accounting for spatial and soil covariates ($R_{adj}^2 = 0.80$) (Table 3, Fig. 5). TSN gains were small and non-significant in conventional ungrazed vineyards (0.01 ± 0.01 Mg TSN ha^{-1} yr^{-1}, $p = 0.162$), but significantly higher in conventional grazed (0.07 ± 0.02, $p = 0.004$), regenerative ungrazed (0.02 ± 0.01, $p = 0.001$), and regenerative grazed vineyards (0.05 ± 0.01, $p < 0.001$). Relative to the conventional ungrazed reference, regenerative grazed vineyards accrued an additional 0.04 ± 0.01 Mg TSN ha^{-1} yr^{-1} ($p < 0.001$) (Table 3, Fig. 5), regenerative ungrazed accrued + 0.01 ± 0.01 ($p = 0.034$), and conventional–grazed accrued + 0.06 ± 0.02 ($p = 0.009$). Within grazed systems, regenerative and conventional vineyards did not differ (–0.02 ± 0.02, $p = 0.420$) (Table 3).

For TSN spatial structure was captured by the 2-D longitude–latitude smooth ($p < 0.001$), and Ca^{2+} saturation exhibited a robust nonlinear effect ($p < 0.001$). Smooths for silt and clay were non-significant ($p = 0.618$ and 0.631).

Plant-Available N (PAN)

Management had a clear effect on PAN after accounting for spatial and soil covariates ($R_{adj}^2 = 0.38$) (Table 3). Years spent under conventional ungrazed management showed no trend in PAN whereas all other management systems showed positive accrual (Table 3). Relative to the conventional ungrazed vineyards, regenerative grazed vineyards accrued an additional 0.94 ± 0.30 kg PAN ha^{-1} yr^{-1} ($p = 0.004$), regenerative ungrazed accrued + 0.48 ± 0.20 ($p = 0.019$), and conventional grazed accrued + 1.49 ± 0.72 ($p = 0.046$) (Table 3). No difference was detected between regenerative and conventional grazed vineyards (Table 3). Spatial location ($p = 0.019$) and Ca^{2+} saturation ($p = 0.034$) also contributed to variation in PAN, while silt ($p = 0.84$) and clay ($p = 0.63$) were not significant. These results demonstrate the importance of integrating grazing for accruing PAN, with both conventional and regenerative grazed systems showing strong positive responses.

3.4.3 Spatial and soil context modify management-driven soil C and N accrual

To assess whether environmental context altered management-driven accrual trajectories (Fig. 5), we fit interaction models allowing management-related slopes to vary across spatial and edaphic gradients (Supplemental Table 5). Geographic position and soil properties explained additional variation in RZA SOC, TSN, and PAN across vineyards. SOC and TSN accrual rates in conventional ungrazed vineyards varied across geographic space ($p \leq 0.018$), while the magnitude of additional gains under regenerative management was modified by soil texture, particularly silt and clay ($p = 0.004–0.019$). Ca^{2+} saturation showed weaker but detectable interactions with some accrual responses ($p \approx 0.03–0.05$). For PAN, the strongest interaction occurred between clay and the accrual response associated with grazing ($p < 0.001$), whereas interactions with Ca^{2+} saturation were weaker and geographic variation in PAN accrual was not supported.

To visualize the environmental context underlying these patterns, we examined the spatial and edaphic components of the GAM predictions (Fig. 6; Supplemental Fig. 5). SOC and TSN showed nonlinear relationships with Ca^{2+} saturation, with generally higher partial effects at higher Ca^{2+} saturation levels, whereas plant-available N declined across the observed Ca^{2+} saturation range (Supplemental Fig. 6). In contrast, marginal smooths for clay and silt were relatively weak (Section 3.4.2), indicating that soil texture primarily modified management-related accrual responses through interactions rather than strong independent effects. Spatial predictions showed localized regions of higher accrual, particularly in vineyards closer to the coast at more northern latitudes and at higher elevations, such as the northern coastal sites in Mendocino County and the more interior, northern, higher-elevation vineyards in Lake County (Fig. 6).

3.4.4 Historical management effects on soil C and N accrual are spatially transferable

We next evaluated whether management histories (conventional ungrazed, conventional grazed, regenerative ungrazed, regenerative grazed) remained predictive of RZA soil carbon and nitrogen accrual beyond local site conditions using spatially buffered cross-validation.

Model performance remained high under spatially independent cross-validation, indicating that management-history effects persisted across broad geographic gradients rather than reflecting localized site effects. For RZA SOC, the GAM with the lowest predictive error included years under regenerative grazed management, along with conventional grazed and ungrazed baselines (5-fold spatially buffered CV: RMSE = 4.85 ± 0.14; $R^2 = 0.63 \pm 0.02$). A more complex model including all years-under-management terms yielded comparable predictive performance (ΔRMSE = 0.20), suggesting that the dominant management signals, particularly the amplification of SOC accrual under regenerative grazing, were robust to alternative specifications of years under management.

A similar pattern was seen for RZA TSN, with significant spatial structure (GAM smooth of latitude and longitude; EDF = 4.27) and strong predictive performance for models incorporating all management trajectories (lowest RMSE = 0.41 ± 0.02; R^2 = 0.46 ± 0.05). Together, these results indicate that although RZA SOC and TSN accrual varied across vineyards in relation to geographic position, years under grazing and regenerative management remained strong and spatially transferable predictors of soil C and N accrual across the study region (Supplemental Table 6).

Per-year effects of management duration on RZA stocks of SOC, TSN, and PAN

Table 3

Per-year effects of management duration on root zone accrual (RZA) stocks of soil organic carbon (SOC) total soil nitrogen (TSN) (0–30 cm), and plant-available nitrogen (PAN) (0–15 cm) in California vineyards. RZA refers to accrued stocks within the 0–30 cm soil depth. Per-year effects were estimated using generalized additive models (GAMs; REML) with linear terms representing years under each management trajectory (conventional ungrazed, conventional grazed, regenerative ungrazed, and regenerative grazed). Slopes represent add-a-year effects (above dividing line) by management system, while contrasts (below dividing line) represent per-year differences between management systems. All models included a two-dimensional spatial smooth (longitude × latitude) and smooth terms for Ca^{2+} saturation, silt, and clay. Confidence intervals and p-values are based on t-distributions using model residual degrees of freedom. Italicized rows and asterisks indicate statistical significance (*p < 0.05; **p < 0.01; ***p < 0.001).

Management trajectories	**Per year**	**95% CI**	**Per 10 years**	**p-value**	**Sig.**
SOC accrual (0–30 cm; Mg SOC ha^{-1} yr^{-1})	Per year	95% CI	Per 10 years	p-value	Sig.
Conventional ungrazed	*0.17 ± 0.08*	*[0.01, 0.34]*	*1.73*	*0.042*	*
Conventional grazed	0.59 ± 0.32	[-0.05, 1.23]	5.89	0.070	
Regenerative ungrazed	*0.34 ± 0.09*	*[0.17, 0.52]*	*3.45*	*< 0.001*	***
Regenerative grazed	*0.55 ± 0.15*	*[0.24, 0.86]*	*5.49*	*< 0.001*	***
Regenerative ungrazed vs conventional ungrazed	0.17 ± 0.09	[-0.01, 0.35]	1.71	0.066	
Regenerative grazed vs conventional ungrazed	*0.38 ± 0.13*	*[0.11, 0.64]*	*3.76*	*0.006*	**
Conventional grazed vs conventional ungrazed	0.42 ± 0.31	[-0.22, 1.05]	4.15	0.192	
Regenerative grazed vs conventional grazed	-0.04 ± 0.32	[-0.68, 0.60]	-0.39	0.902	
TSN accrual (0–30 cm; Mg TSN ha^{-1} yr^{-1})	**Per year**	**95% CI**	**Per 10 years**	**p-value**	**Sig.**
Conventional ungrazed	0.01 ± 0.01	[-0.00, 0.02]	0.08	0.162	
Conventional grazed	*0.07 ± 0.02*	*[0.02, 0.11]*	*0.67*	*0.004*	**
Regenerative ungrazed	*0.02 ± 0.01*	*[0.01, 0.03]*	*0.22*	*0.001*	**
Regenerative grazed	*0.05 ± 0.01*	*[0.03, 0.07]*	*0.49*	*< 0.001*	***
Regenerative ungrazed vs conventional ungrazed	*0.01 ± 0.01*	*[0.00, 0.03]*	*0.14*	*0.034*	*
Regenerative grazed vs conventional ungrazed	*0.04 ± 0.01*	*[0.02, 0.06]*	*0.41*	*< 0.001*	***
Conventional grazed vs conventional ungrazed	*0.06 ± 0.02*	*[0.02, 0.10]*	*0.59*	*0.009*	**
Regenerative grazed vs conventional grazed	-0.02 ± 0.02	[-0.06, 0.03]	-0.18	0.420	
PAN (0–15 cm; kg N ha^{-1} yr^{-1})	**Per year**	**95% CI**	**Per 10 years**	**p-value**	**Sig.**
Conventional ungrazed	-0.08 ± 0.19	[-0.46, 0.29]	-0.83	0.659	
Conventional grazed	1.41 ± 0.73	[-0.07, 2.88]	14.05	0.062	
Regenerative ungrazed	*0.40 ± 0.20*	*[0.01, 0.80]*	*4.00*	*0.047*	*
Regenerative grazed	*0.86 ± 0.35*	*[0.14, 1.57]*	*8.55*	*0.020*	*
Regenerative ungrazed vs conventional ungrazed	*0.48 ± 0.20*	*[0.08, 0.88]*	*4.83*	*0.019*	*
Regenerative grazed vs conventional ungrazed	*0.94 ± 0.30*	*[0.32, 1.55]*	*9.38*	*0.004*	**
Conventional grazed vs conventional ungrazed	*1.49 ± 0.72*	*[0.03, 2.94]*	*14.88*	*0.046*	*
Regenerative grazed vs conventional grazed	-0.55 ± 0.74	[-2.05, 0.95]	-5.50	0.463	

4. Discussion

Across 49 vineyards spanning diverse soils and climates in Northern and Central California, sheep grazing emerged as one of the most influential practices associated with higher soil organic matter (SOM) pools. When integrated within regenerative systems characterized by reduced tillage, permanent ground cover, and minimized synthetic inputs, soil carbon and nitrogen accrual rates exceeded those of conventional ungrazed vineyards by more than threefold. These results support livestock integration as a keystone practice within regenerative perennial systems, accelerating carbon and nitrogen accumulation while interacting with geographic and edaphic constraints that shape SOM storage.

Regenerative management context mediates grazing effects on SOM accrual

Within SOM pools, results showed complex interactions among co-occurring management practices. Divergence between RF and GAM results reflects fundamental differences in how each method attributes importance: RF importance is sensitive to correlations among co-occurring practices and may underestimate the contribution of any single practice, whereas GAM marginal effects estimate each predictor's independent association after accounting for spatial, edaphic, and co-management covariates. Convergence between both approaches therefore provides the strongest evidence for a practice's influence on soil carbon and nitrogen accrual. After accounting for vineyard location and soil covariates model approaches converged toward grazing as a strong positive driver of total soil nitrogen (TSN), particulate carbon (POC) and nitrogen (PN), and mineral-associated nitrogen (MAN). No-till management in the tractor row showed a reliable positive effect on soil organic carbon (SOC), while permanent soil cover was positively associated with TSN. These practices likely form a core management toolkit aligning with perennial cropping literature emphasizing continuous cover (84, 85), reduced disturbance (86–88), and biologically mediated nutrient cycling (27, 89). Other regenerative practices showed more variable effects across SOM pools. Organic inputs and the avoidance of synthetic insecticides ranked highly in RF models but showed weaker support in GAM analyses that evaluated their independent effects. Organic inputs were positively associated with SOC and mineral-associated organic carbon (MAOC) pools in RF models, in line with their role as external carbon and nitrogen inputs (90, 91). Avoidance of synthetic insecticides may influence soil processes indirectly through changes in soil food webs, detritivore activity, and nutrient cycling (92, 93). These results highlight grazing as a leverage practice shaping soil carbon and nitrogen accrual while emphasizing the importance of stacking multiple practices to build a regenerative management system.

Integrating grazing within regenerative systems was associated with higher carbon and nitrogen accrual rates in working vineyards, supporting emerging evidence from other cropping systems (35, 94). Winter cover cropping was present in all vineyards, and approximately 50% of conventionally classified vineyards (< 4 regenerative practices) utilized compost and no-till in the tractor row. Together, these practices likely established a baseline trajectory of SOM accrual in conventional sites (87, 95, 96), reflecting the widespread adoption of soil health practices across California viticulture. Regenerative management (≥ 4 regenerative practices) and grazing accelerated these gains greater than threefold for carbon accrual and fivefold for nitrogen accrual (Table 3). Although the conventional grazed carbon accrual rate was not statistically significant (0.59 ± 0.32 Mg SOC ha^{-1} yr^{-1}, $p = 0.070$), its point estimate was comparable to regenerative grazed systems (0.55 ± 0.15 Mg SOC ha^{-1} yr^{-1} f, $p < 0.001$), with wider confidence intervals potentially reflecting the smaller number of conventional grazed sites and their compressed range of management years (Supplemental Fig. 1B) rather than an absence of a grazing effect. Accrual rates were lower than those reported in a vineyard meta-analysis (87) and a literature review of vineyard soil carbon dynamics (88), but were comparable to global SOC potential estimates (8) and a meta-analysis of conservation agriculture in Mediterranean field crops (97). Given estimated baseline stocks of approximately 39 Mg SOC ha^{-1} (0–30 cm), both conventional ungrazed and regenerative grazed trajectories met or exceeded the Four Per Thousand target of a 0.4% annual stock increase (8, 98). Similar trends for TSN and plant available nitrogen (PAN) further reinforce the significant role of grazing, with grazed systems accruing more N regardless of regenerative classification. Considering that working farms often exhibit lower accrual rates than research plots due to management variability, legacy effects (42, 43), and lower organic amendment application rates (46) these results demonstrate meaningful, scalable mitigation potential on commercial operations.

Grazing alleviates nitrogen constraints on soil carbon accrual

Since SOM maintains a constrained C:N ratio of approximately 12:1, long-term carbon accrual is fundamentally regulated and limited by nitrogen availability (33). Mineral-associated organic matter (MAOM) forms predominantly through microbial processing of nitrogen-rich substrates, with nitrogen-rich inputs increasing microbial carbon use efficiency and necromass production. Nitrogen availability is therefore a specific constraint on MAOC formation, extending beyond its important role in regulating bulk SOM accrual (99–101). Grazing appeared to alleviate nitrogen constraints on SOM formation including the more stable mineral-associated SOM pools, while reducing synthetic fertilizer dependence (Table 1). Grazed vineyards exhibited higher TSN, MAN, PN, and PAN alongside elevated SOC and MAOC. This pattern aligns with grazing-driven enhancement of nitrogen cycling through manure inputs, stimulated root turnover, and increased microbial activity (21, 27, 35). Increases in PAN can present agronomic trade-offs in wine grape systems, where excessive nitrogen availability may stimulate vine vigor and yields, while possibly reducing grape quality. However, the nitrogen gains observed here were gradual (approximately 1 kg N ha^{-1} yr^{-1}), suggesting incremental improvements in nitrogen availability mediated through microbial activity and soil organic matter turnover versus direct fertilizer inputs. Over time, these gains can be agronomically meaningful without driving excessive vine vigor or reduced grape quality, equating

to approximately 10 kg N ha^{-1} of additional PAN over a decade. In wine-grape systems that require relatively low nitrogen inputs, these gains can help sustain yields, support microbial turnover, and reduce dependence on synthetic nitrogen. Regarding concerns about increased nitrous oxide emissions, other research in California vineyards has shown episodic N_2O pulses following grazing, yet cumulative seasonal emissions did not exceed those of non-grazed soils (29). These findings suggest that grazing-driven nitrogen cycling may provide a biologically sustainable pathway for SOC sequestration by aligning carbon inputs with the nutrient requirements for stable SOM formation while helping offset external fertilizer inputs and remaining compatible with vineyard fertility objectives.

Spatial and edaphic gradients shape SOM accrual across vineyards

Spatially buffered cross-validation indicated that models incorporating management histories and soil covariates remained predictive for SOC and TSN stocks when evaluated across geographically independent vineyards, suggesting that observed management–accrual relationships extend beyond localized site conditions. Against this broader spatial backdrop, environmental gradients shaped the magnitude and spatial distribution of soil carbon and nitrogen gains across vineyards.

Carbon and nitrogen accrual were concentrated in the upper 30 cm of soil reflecting rooting depth and microbial activity patterns in cropping systems (47, 64, 102). Across vineyards, geographic position and soil properties were among the strongest predictors of SOM pools. Latitude, longitude, and soil texture reflect broader environmental gradients across California vineyards, with northern, higher-elevation, and coastal regions receiving greater winter rainfall and cooler summers than warmer southern and inland environments (103, 104). For example, coastal vineyards in Mendocino County represent the cooler northern coastal portion of the study region, whereas sites in Lake County occur at higher elevations within the northern interior. These conditions support greater biomass production and slower decomposition rates, increasing the potential for soil organic matter accumulation (34). In line with this pattern, higher soil carbon and nitrogen accrual were found in northern coastal and higher-elevation vineyards (Fig. 6). Soil texture also influenced SOM accumulation, consistent with finer-textured soils providing greater mineral surface area for organic matter stabilization (36). Ca^{2+} saturation was also an important predictor, aligning with calcium-mediated aggregation and organo-mineral stabilization (37, 39), processes most pertinent in soils with pH > 6.5 (conditions present across all vineyards in this study). Interaction models further indicated that management responses were context dependent, with soil texture, Ca^{2+} saturation, and geographic position modifying accrual across management histories. Collectively, these results suggest that while management practices influence the rate of soil carbon and nitrogen accrual, environmental gradients shape where and how strongly these gains occur across vineyards.

Limitations

Bulk density was estimated using a pedotransfer function rather than measured directly or calculated using equivalent soil mass, which may introduce additional uncertainty in absolute stock values. However, pedotransfer functions are widely used in multi-site studies and provide a reliable basis for relative comparisons across sites. Given the snapshot, space-for-time design, where pre-treatment baselines and repeated measures of the same soil mass were unavailable, the Franzluebbers depth-distribution framework provides a practical alternative by estimating baseline conditions from the vertical soil profile. This approach enables robust comparison of relative accrual across sites, while also supporting construction of chronosequences across farms with differing management histories (47–49). However, space-for-time substitution introduces uncertainty associated with legacy effects, unmeasured heterogeneity, and residual spatial structure. By deriving site-specific baselines from each vineyard's depth profile, the Franzluebbers framework partially addresses the assumption that sites shared comparable starting conditions. Nevertheless, legacy land use effects and unmeasured pre-management heterogeneity cannot be fully resolved without longitudinal baseline data. Although spatially buffered cross-validation supports the generality of observed patterns, causal inference remains limited by the observational design. Further, stacked management practices constrain the ability to disentangle independent effects of individual practices, highlighting the need for complementary long-term longitudinal experiments.

5. Conclusions

These findings illustrate how integrating livestock into perennial cropping systems, especially when embedded within regenerative systems that combine multiple practices, can align soil carbon sequestration, nitrogen cycling, and agricultural production to support more resistant and resilient multifunctional agroecosystems. While shaped by geographic and edaphic properties, grazing integrated alongside regenerative management provides a biologically grounded pathway to increase soil carbon and nitrogen stocks, meet or exceed international benchmarks, and enhance co-benefits, reducing reliance on synthetic nitrogen and herbicides in perennial systems. Since models incorporating management histories retained predictive capacity under spatial cross-validation, regenerative grazing may generate broadly transferable soil carbon and nitrogen benefits across Mediterranean perennial agricultural systems. In addition to soil health benefits, incorporating sheep into vineyard systems can enhance agroecosystem multifunctionality by supporting livestock-based food production alongside wine grapes with reduced vegetation management intensity. Livestock integration can also strengthen agroecosystem resilience and resistance through reduced reliance on external inputs, expanded forage resources and wildfire risk mitigation via reduced fuel loads. For growers and

policymakers, these results support incentive structures that reward long-term adoption of integrated crop–livestock and regenerative systems tailored to local soils and climates as a realistic pathway for climate mitigation and agricultural resilience.

Declarations

Availability of data and materials

The datasets and R code supporting the conclusions of this article are available in the USDA Ag Data Commons repository, [https://agdatacommons.nal.usda.gov/].

Competing interests

The authors declare no competing interests.

Funding sources

This work was supported by Western SARE Research and Education project SW23-949 to AG and JL.

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Consent to publish

Consent to Publish declaration: not applicable

Ethics and Consent to Participate

Grower surveys were reviewed by the UC Davis Institutional Review Board (IRB ID: 2022821-2) and determined exempt under Category 2 (June 30, 2023). Sampling was conducted on commercial farm properties with grower consent.

Acknowledgements

We sincerely thank the team for supporting field and lab work: Max Carpenter, Joshua Estes, Elizabeth Coon, Aldo Vorkapich, Emily Chou, Laura Johnson, Clarissa Arnaudo, Kyle Moeller, Thea Wagner, Noah Homsy-King, Laura Caruthers, Elsa Robelot, Dr. Matt Jones, Dr. Stephen

Robertson, Greg Richardson, Christina Lind, Amy Heibult, Tia Busenitz, Mia Werger, Lynnette Miller, Dr. Ryan Schmid, Daniel Pecenka, Felicia Rain, Gordan Gallant, Olivia Torbet, William Hillery, Morgan Pederson, Cassandra Koel, Jay Skaar, Sydney Golding, Kelly Clinton, Dan Macina, Dr. Kelton Welch.

We also wish to acknowledge the larger grant project team for their insights and support: Dr. Brittney Goodrich, Dr. Elizabeth Forrestel, Sara Tiffany and Layla Aguilar (Community Alliance with Family Farmers), Miguel Garcia and Lucas Patzek (Napa RCD), Rebecca Burgess (Fibershed), Kelly Mulville, Greg Richardson, Colden Proe, and Elaine Patarini (Paicines Ranch), Clay Shannon and Tim Griffin (Shannon Vineyards), Maria Zumkeller (Lange Twins), Natalie Birch and Sophia Bates (Navarro/ Pennyroyal), Jamie and Robert Irwin (Kaos). We thank the following graziers for their observations: Chris Maschauer (Napa Vineyard Sheep Grazing), Christian Cain (Perennial Grazing). Finally, we thank all the vineyards, vineyard workers, and managers who supported this project and allowed us to sample on their property.

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Figures

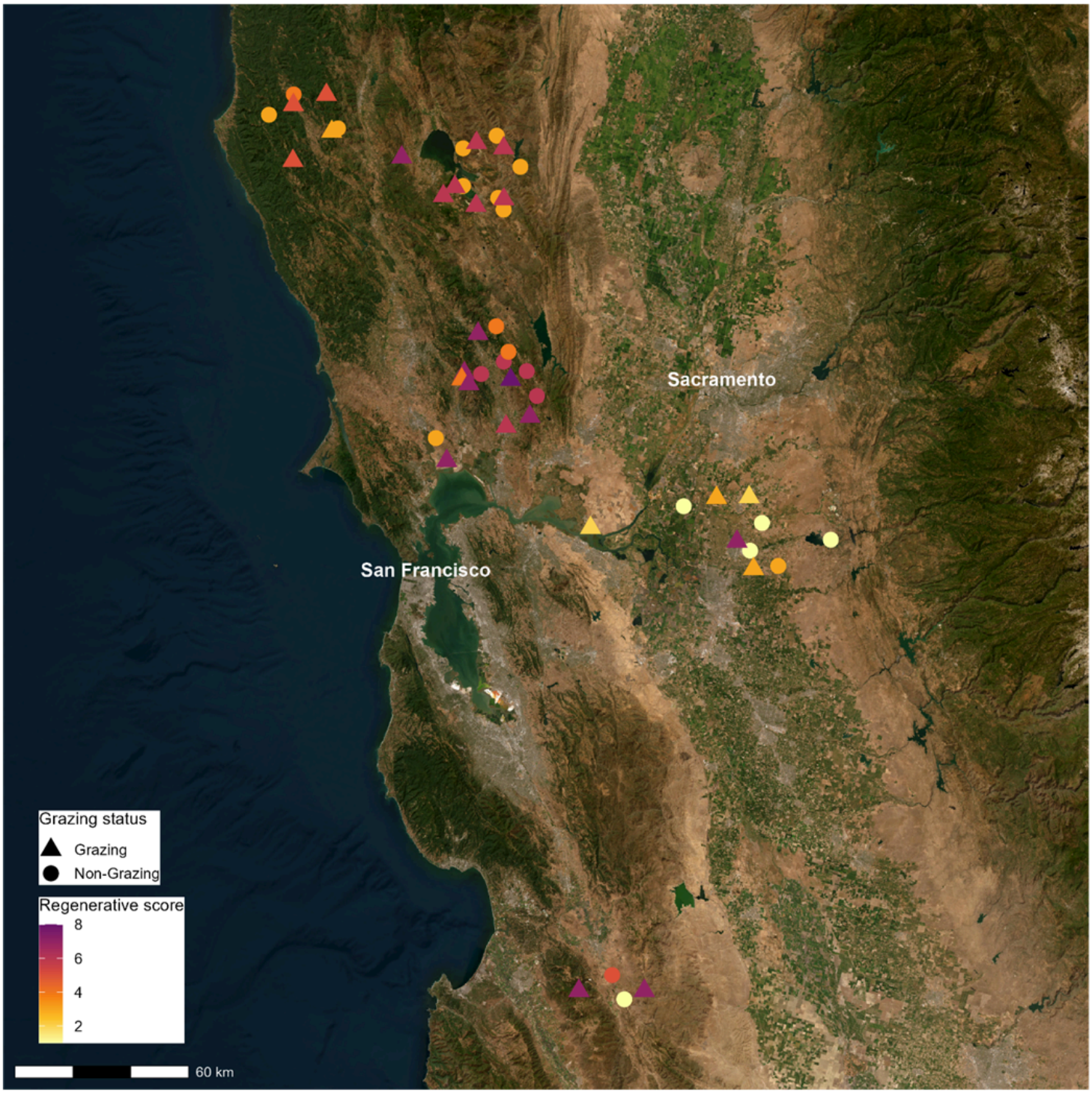


Figure 1

Map of the forty-nine wine grape vineyard sites sampled across Northern and Central California. Sites were selected as paired vineyards with and without sheep grazing located within 20 km of each other on similar soils and grape varieties. Both grazed and ungrazed sites spanned a conventional–regenerative co-management gradient quantified by a regenerative score. Vineyards received a score of 1 for each regenerative practice adopted or conventional practice avoided, yielding a maximum possible regenerative score of 8 (Table 1). Practices earning a score of 1 included the use of organic amendments, no-till, winter cover crops, and integration of grazers. Use of synthetic fertilizers, herbicides, insecticides, and fungicides received a score of 0, whereas avoiding these inputs received a score of 1. Vineyards with scores ≥4 were classified as regenerative and those with scores <4 as conventional (9) *. Grazed sites are indicated by triangles and non-grazed sites by circles, with the color gradient*

indicating each site's regenerative score. Point locations are slightly jittered (displaced) to avoid overlapping. See table 1 for the distribution of management practices among the different vineyards.

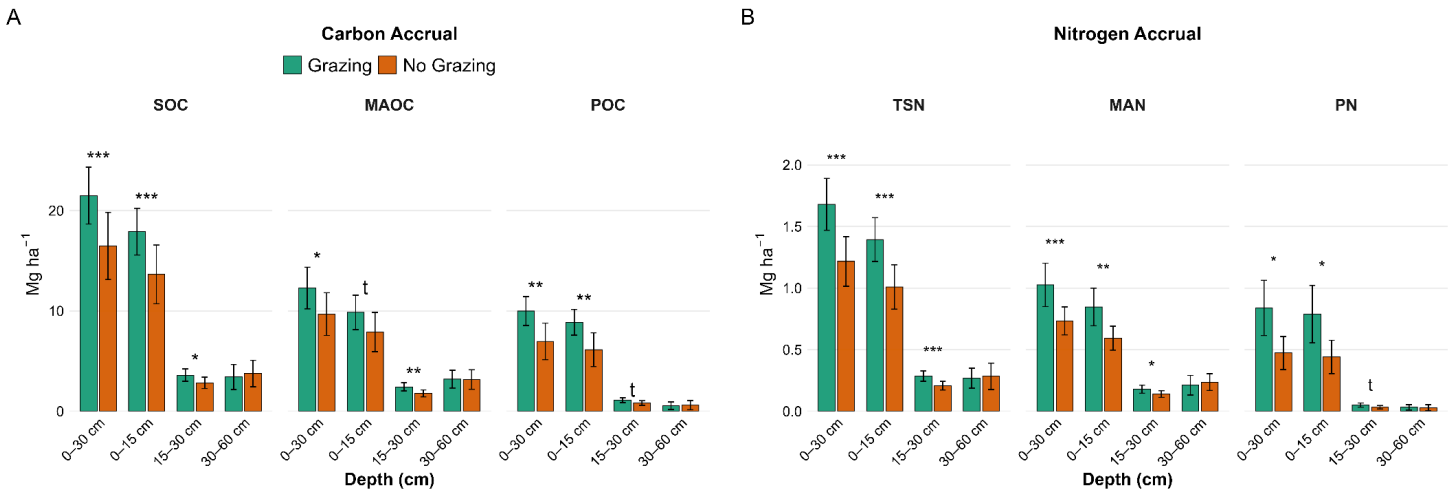


Figure 2

*Mean accrued stocks of soil carbon and nitrogen across soil organic matter (SOM) pools (soil organic carbon [SOC], mineral-associated organic carbon [MAOC], particulate organic carbon [POC], total soil nitrogen [TSN], mineral-associated nitrogen [MAN], particulate nitrogen [PN]) and soil depths (0–30 cm, 0–15 cm, 15–30 cm, and 30–60 cm) in established California wine grape vineyards as a function of grazing management (vineyards with ≥3 years of grazing vs ungrazed vineyards). The 0–30 cm interval represents Root Zone Accrual (RZA). Carbon (A) and nitrogen (B) accrued stocks were calculated as the difference between measured stocks and estimated baseline stocks. Error bars represent 95% confidence intervals (CIs) and means and CIs are shown using untransformed values. Significance levels are indicated as follows: *** < 0.001, ** < 0.01, * < 0.05, t ≥ 0.05 < 0.10. Reported p-values correspond to the coefficient for established grazing management in generalized additive models that accounted for spatial and edaphic variables.*

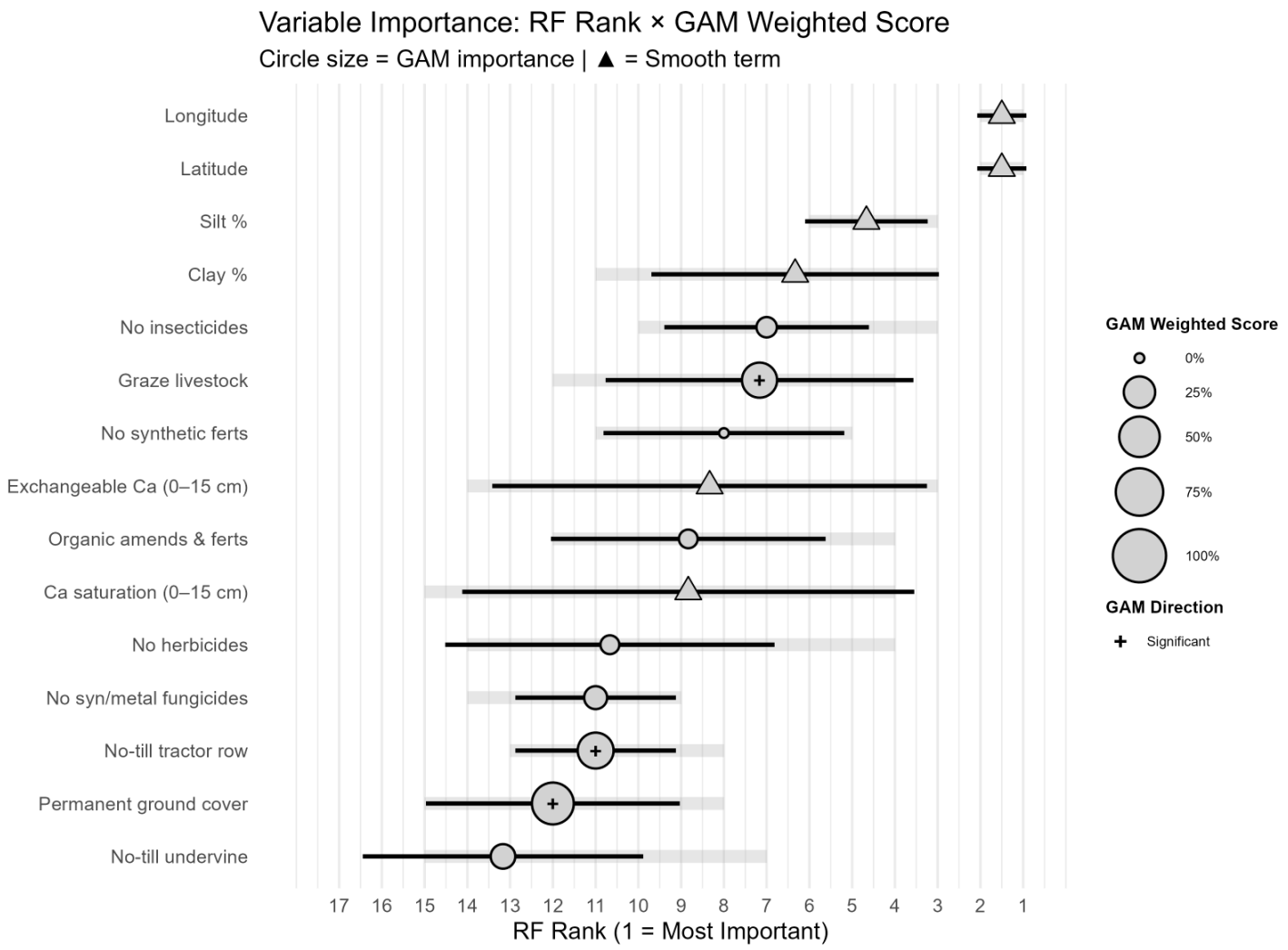


Figure 3

Mean cross-pool Random Forest (RF) and generalized additive model (GAM) importance of vineyard management and soil–spatial predictors across six soil root zone accrual (RZA) carbon and nitrogen pools (soil organic carbon [SOC], mineral-associated organic carbon [MAOC], particulate organic carbon [POC], total soil nitrogen [TSN], mineral-associated nitrogen [MAN], particulate nitrogen [PN]; 0–30 cm) in California wine grape vineyards. Points show mean RF importance ranks (1 = highest importance) with 95% confidence intervals (black lines) and best–worst rank ranges (grey lines); circles denote management variables and triangles denote soil or spatial smooth terms. Circle size reflects the GAM Weighted Score (Σ AICc weight × 1[p < 0.05]) normalized across pools Directional markers ("+") indicate predictors with consistent positive effects across pools, determined using unweighted meta-analytic p-values (p < 0.1) with ≥0.75 sign dominance, assigned when criteria were met in at least three pools and accounted for ≥50% of total weighted directional support. Predictors not meeting these criteria are shown without a directional marker. No predictors met the criteria for consistent negative effects across pools.

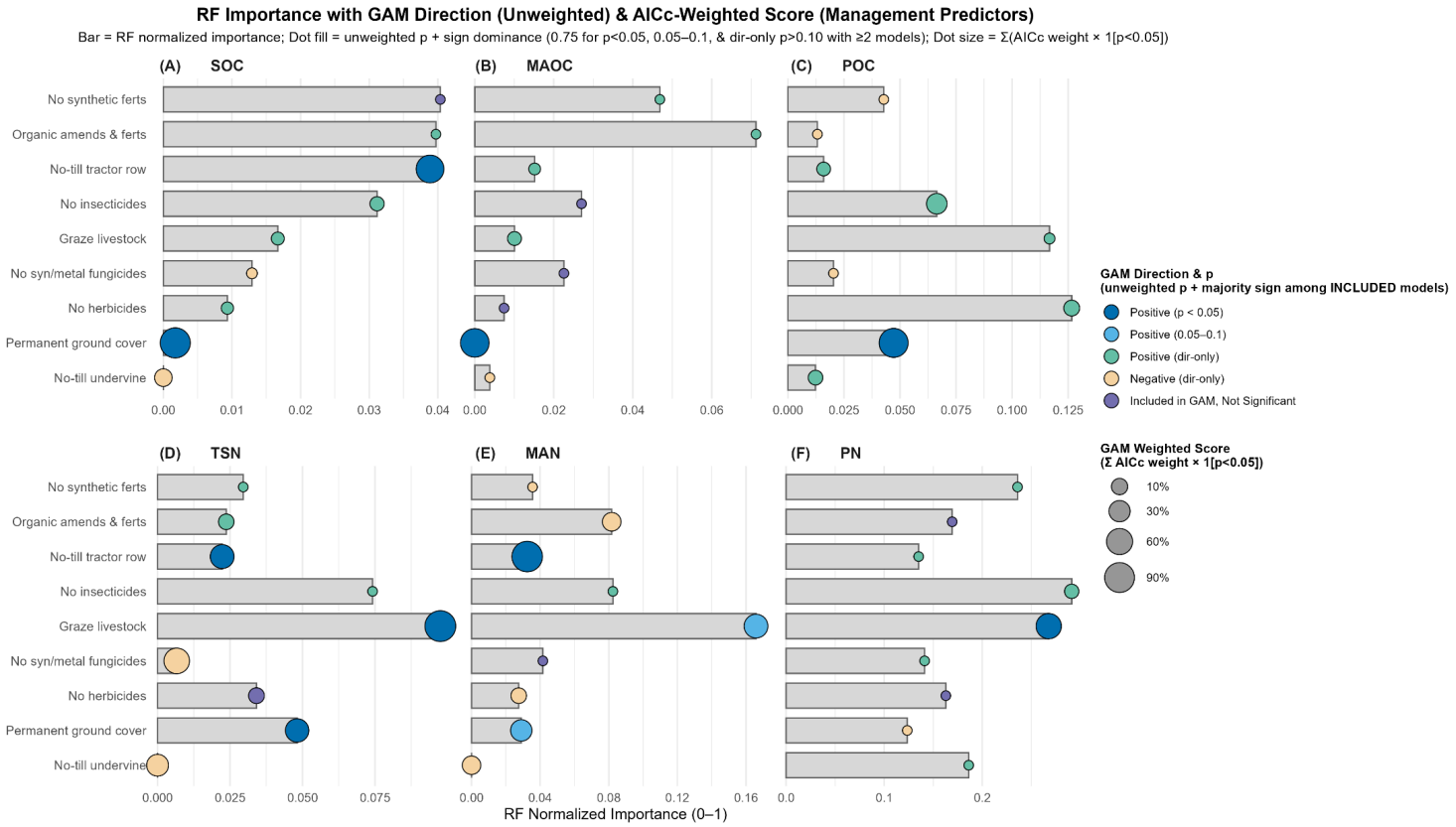


Figure 4

Management predictors for each of the six root zone accrual (RZA) soil organic matter (SOM) pools (soil organic carbon [SOC], mineral-associated organic carbon [MAOC], particulate organic carbon [POC], total soil nitrogen [TSN], mineral-associated nitrogen [MAN], particulate nitrogen [PN]; 0–30 cm) in California wine grape vineyards. Together, this RF–GAM integration combines predictive importance with directional statistical evidence for each SOM pool. The bars show Random Forest (RF) normalized importance derived from permutation importance (%IncMSE) and rescaled within each SOM pool (0–1) to facilitate comparison of predictor importance across pools. Dot color indicates generalized additive model (GAM) effect direction and evidence tier derived from unweighted meta-analytic p-values and sign dominance across GAMs within ΔAICc ≤ 7. Dark blue/orange denote positive/negative effects with p < 0.05; lighter blue denotes marginal effects (0.05–0.10); teal/cream denote direction-only cases where p > 0.10 but ≥0.75 of included models (≥2) share the same sign (dir-only); purple indicates predictors included in ΔAICc ≤ 7 GAMs but lacking consistent directional evidence across models. Dot size reflects the GAM Weighted Score (Σ AICc weight × 1[p < 0.05]), summarizing the proportion of AICc-supported models in which the predictor was significant.

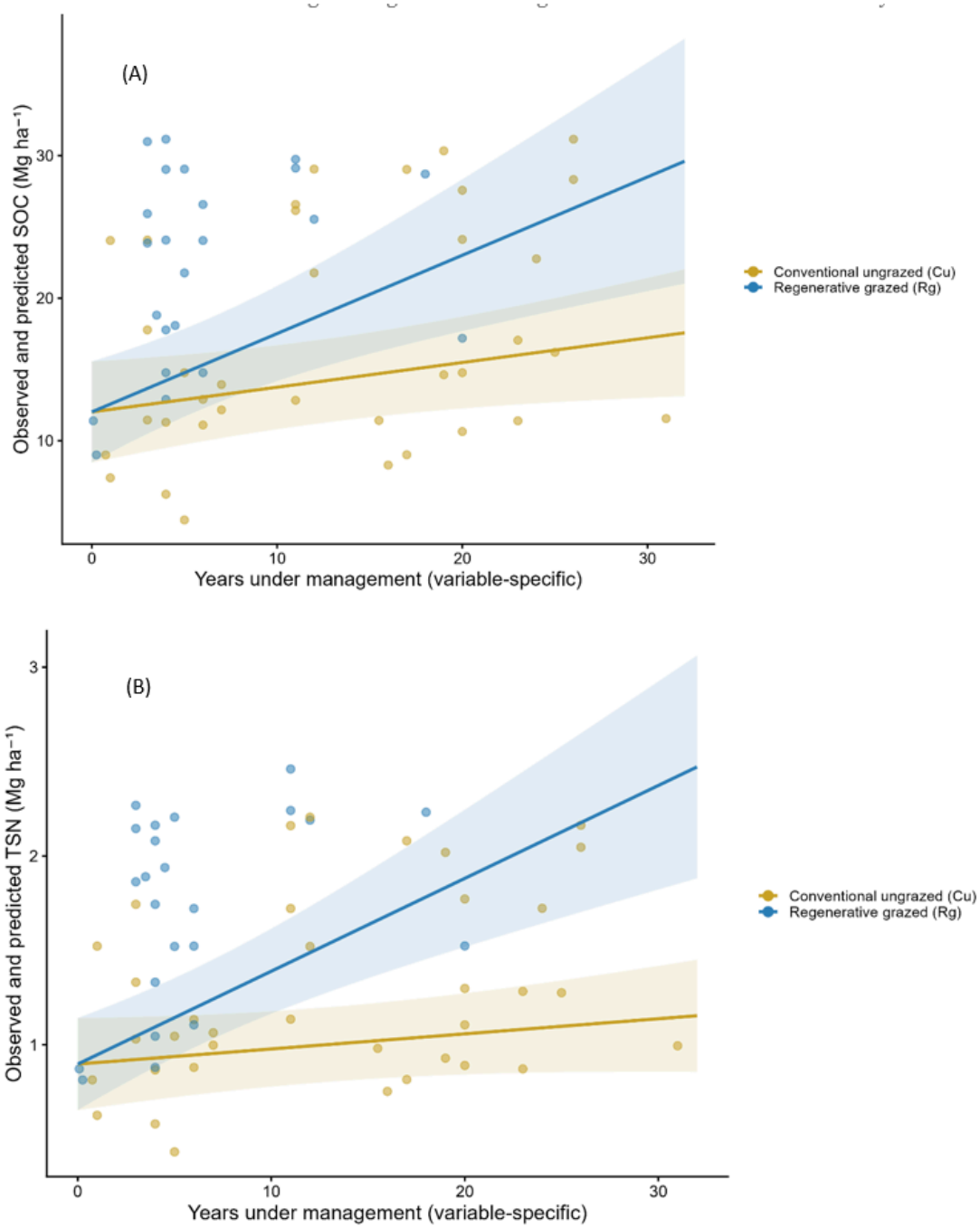


Figure 5

contrasting management histories in California vineyards, as predicted by generalized additive models (GAMs). The model simultaneously accounted for time under all four management combinations (conventional ungrazed, conventional grazed, regenerative ungrazed, regenerative grazed), allowing estimation of independent per-year effects for each system. For visualization clarity, only the most contrasting conventional–ungrazed trajectory (gold) and the regenerative–grazed trajectory (blue) are shown. Lines show modeled changes over time while holding spatial and edaphic covariates at their median values. Shaded ribbons represent 95% confidence intervals, and points show observed farm-level values; farms with zero years in the displayed management category were omitted. Although total management years were mean-centered during model fitting, predictions are plotted on the original year scale for interpretability, and slopes represent per-year effects relative to the centered conventional–ungrazed reference condition.

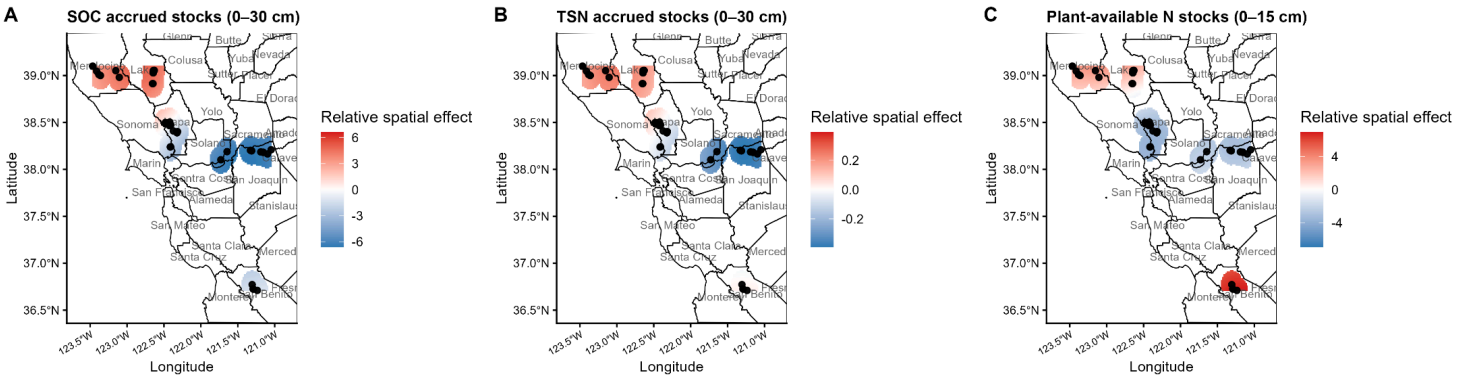


Figure 6

Spatial patterns in model-predicted soil C and N accrual across vineyards. Spatial predictions from generalized additive models (GAMs) showing geographic variation in root zone accrued (RZA) soil organic carbon (A; SOC; 0–30 cm), total soil nitrogen (B; TSN; 0–30 cm), and plant-available nitrogen (C; PAN; 0–15 cm) across vineyard locations. Colors represent the spatial component of the model predictions after accounting for management history duration (conventional ungrazed, conventional grazed, regenerative ungrazed, regenerative grazed) and edaphic covariates included in the models. Warmer colors indicate areas where predicted accrual is higher relative to the model mean, whereas cooler colors indicate lower predicted accrual. Points show sampled vineyard locations, and county boundaries are shown for geographic reference. Color scales are centered at zero and scaled independently for each response to reflect differences in magnitude among SOC, TSN, and PAN spatial patterns.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable5GAMExploratoryInteractions.xlsx
- SupplementalTable3GAMModelResults.xlsx
- SupplementalMaterialsGrazinginvineyardsfullblanksurvey.pdf
- 20260407SupplementaltablesfiguresmethodsCBalancemgmt.docx
- SupplementalRpackagereferences.docx