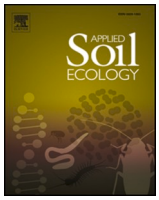
Applied Soil Ecology 215 (2025) 106419

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Research paper

Effects of grazing exclusion and pasture management on soil microbial communities in Californian irrigated pastures

Madison L. Morris [a,*,1], Danny J. Eastburn [b], Leslie M. Roche [b], Josh Davy [c], Morgan Doran [c], Betsy Karle [c], David Lile [c], Tracy Schohr [c], Laura Snell [c], Dan Macon [c], Grace Woodmansee [c], María Touceda-Suárez [a], Albert Barberán [a]

[a] Department of Environmental Science, University of Arizona, Tucson, AZ 85721, USA
[b] Department of Plant Sciences, University of California, Davis, CA 95616, USA
[c] University of California Cooperative Extension, Davis, CA 95616, USA

ARTICLE INFO

Keywords:
Livestock grazing
Irrigated pastures
Soil microbial diversity
Amplicon sequencing

ABSTRACT

Grazing lands in California and across the globe are increasingly challenged to meet rising livestock product demands while simultaneously balancing diverse stakeholder and land management goals. An increasing focus on the soil health of grazed landscapes has enhanced our understanding of grazing impacts on sustainable agroecosystems. However, the scientific literature is limited on how the microbial community, as a component of soil health, responds to grazing, water, and soil nutrient management in irrigated pastures. We deployed a cross-sectional survey across 24 California irrigated pastures spanning multiple climate regimes and active management strategies. We established and maintained grazing exclosures for two years and collected soil samples from rested and grazed plots within each irrigated pasture. We used 16S rRNA and ITS amplicon sequencing to analyze soil bacteria and archaea, and soil fungi, respectively. Microbial diversity and community composition were not affected by grazing rest or management, but fungal Shannon diversity was significantly impacted by total nitrogen (TN; mixed linear effect model, $p = 0.044$). Bacterial/archaeal and fungal community compositions were significantly different between pastures (PERMANOVA; $R^2 = 0.78$, $p < 0.001$ for 16S; $R^2 = 0.71$, $p < 0.001$ for ITS). Soil properties were also significantly different between pastures (PERMANOVA, $R^2 = 0.98$, $p = 0.001$) and differed to a lesser extent based on the level of grazing, irrigation, and nutrient management efforts ($R^2 = 0.022$, $p = 0.022$). We found trends among microbial functional groups in response to grazing, but none of the impacts were statistically significant after accommodating false discovery errors. These results support a growing body of evidence that soil microorganisms are variably influenced by livestock grazing and are largely shaped by local vegetation and soil characteristics, both of which can vary based on geography and land management legacies.

1. Introduction

Across California's grazinglands and worldwide, the livestock grazing industry is increasingly challenged to meet rising livestock product demands while simultaneously balancing diverse stakeholder goals and the supply of multiple ecosystem services. Spanning more than 200,000 ha in California, irrigated pastures, which are generally more intensively managed than rangeland systems, play a crucial role in providing an essential forage during the summer dry period, when the lower elevation native grazed rangeland ecosystems are dormant. These lands sustain a livestock product industry valued at $12.3 billion, ranking as the state's fifth largest agricultural commodity (California Department of Forestry and Fire Protection, 2018; California Department of Food and Agriculture, n.d). The industry consists of 12,000 California ranchers, whose livelihoods rely on the health and productivity of grazing lands in the face of increasing regulatory and environmental stressors (California Department of Forestry and Fire Protection, 2018; Roche et al., 2015a, 2015b; Roche, 2016). Irrigated pastures have the potential, as analogs to the wetland ecosystems lost over the last century, to simultaneously supply various ecosystem services, such as animal-based protein production, wildlife habitat, and groundwater recharge (Huntsinger et al., 2017; Shapero et al., 2017). Moreover, in Western North America, these

* Corresponding author.
E-mail address: madmorris@ucdavis.edu (M.L. Morris).
[1] Present address: Department of Land, Air and Water Resources, University of California, Davis, CA 95616, USA.

https://doi.org/10.1016/j.apsoil.2025.106419
Received 11 June 2025; Received in revised form 4 August 2025; Accepted 24 August 2025
Available online 29 August 2025
0929-1393/Published by Elsevier B.V.

systems are often located on marginal soils unsuitable for other types of intensive and more profitable agricultural operations. Soil health underpins a suite of ecosystem services, promotes resilience, and sustains the livelihoods of ranchers while supplying a multitude of benefits (i.e. climate regulation) to society. Understanding how the management of grazing, irrigation, and fertilization activities affect soil health in pasture soils is critical to safeguarding grazingland resilience and the provisioning of ecosystem services (Derner et al., 2018).

Livestock grazing can influence agroecosystems in ecologically desirable and undesirable ways through multiple mechanisms, including but not limited to defoliation and physical damage to plants, trampling and compaction of soil, and redistribution of nutrients via digestion and excretion of organic matter. Understanding the ecological effects of livestock grazing requires a focus on pasture management variables such as the temporal and spatial distribution of animals, the timing and frequency of use, and stocking rate (animal units per hectare) relative to carrying capacity and forage production. Grazing systems are inherently dynamic and span a spectrum of diverse management strategies (Roche et al., 2015a, 2015b); despite this variability, some studies have associated well-managed grazing with positive ecological effects, such as enhanced seed dispersal (Plue and Cousins, 2018; Couvreur et al., 2004), increased ecosystem C (Holland et al., 1992; Shao et al., 2013; Byrnes et al., 2018), increased plant species richness (Rusch et al., 1997), and reduced abundance of invasive plants (Demeter et al., 2021; Porensky et al., 2020; Rhodes et al., 2021; Davy et al., 2015). Previous work has also clearly shown that unmanaged or excessive livestock grazing can severely reduce plant cover, increasing erosion risks and leading to ecosystem degradation (Yong-Zhong et al., 2005; Steinfeld and Wassenaar, 2007; Schönbach et al., 2011). Additionally, physical trampling by livestock can cause soil compaction, as evidenced by reduced infiltration rates and higher soil bulk density (Zhou et al., 2010, Byrnes et al., 2018).

Pasture management variables such as irrigation and fertilization critically introduce new inputs to the soil system to facilitate forage production, but may also cause unintended impacts on soil properties and the microbial community. In an experimental pasture receiving long-term (50+ years) P fertilization, researchers observed an increase in TN and nitrate and a decrease in soil pH, with a significant correlation between soil properties and soil moisture and temperature as well (Touhami et al., 2023). Bacterial and fungal community structures have also demonstrated a response to fertilization in grazed systems (Wakelin et al., 2012), with an increase in fungal richness detected when fertilizer was applied to a continuously grazed pasture (Wakelin et al., 2009). Neutral and positive effects of system intensification (fertilization and increased stocking rate) on the bacterial capacity for nitrification and denitrification were further detected in grazed pastures (Wakelin et al., 2009). Similarly, irrigation management can affect soil chemical properties and microbial communities. Irrigated dairy pastures in one study experienced an increase in biomass production, N outputs, and N leaching compared to non-irrigated pastures (Graham et al., 2022). Within the microbial communities, a study comparing 28 irrigated and non-irrigated pastures found that irrigation led to different microbial community structures, increased relative abundance of gram-negative bacteria, and a decreased fungal:bacterial ratio, leading researchers to conclude that irrigation facilitates rapid, bacteria-dominated cycling in soils (Lambie et al., 2021). The diverse effects of pasture management factors such as fertilization and irrigation must be considered when assessing soil properties in grazed ecosystems.

Soil microbial communities can be important indicators of soil health and plant productivity (Fierer et al., 2021; Van der Heijden et al., 2008). For instance, in tropical grassland pastures, soil microorganisms facilitate the return of nitrogen (N), phosphorus (P), and organic carbon (C) into plant-available forms contributing to soil fertility (Dubeux et al., 2007). When compared to rested enclosures, continuous grazing has been linked with negative soil health effects such as decreased SOM, TN, and microbial biomass (Liu et al., 2016). Alternatively, in a meta-analysis spanning multiple grazing intensities, grazed grasslands had higher soil net N mineralization and N nitrification compared to ungrazed lands, and light grazing specifically contributed to soil C and N sequestration (Zhou et al., 2017). Another meta-analysis found that light and moderate grazing intensities had no significant effect on soil microbial communities, while heavy grazing intensities were associated with 14–28 % decreases in soil bacterial, fungal, and total microbial numbers (Zhao et al., 2017). Similarly, continuous overgrazing has been associated with reduced microbial activity in arid savannahs (Abril and Bucher, 1999) and semi-arid grasslands (Yong-Zhong et al., 2005). In contrast, 1–2 years of rest from moderate grazing in alpine pastures led to increases total microbial biomass, with grazing rest particularly increasing fungal and arbuscular mycorrhizal biomass (Zhang et al., 2025). More research is needed to better understand how grazing affects belowground microbial communities in order to improve soil health on grazing lands and ultimately support system function, resilience, and sustainability (Derner et al., 2018). Previous research suggests that intensive grazing can reduce microbial diversity and alter microbial community composition, with further variability due to pasture management decisions. We hypothesized (a) grazed plots would be associated with reduced soil microbial diversity compared to plots rested for 2 years, (b) grazing exclusion would significantly alter soil microbial community compositions, (c) nitrifying bacteria and mycorrhizal fungi would be more abundant in grazed and ungrazed plots, respectively, and (d) higher effort irrigated pasture management strategies would favor higher microbial diversity.

To test these hypotheses, we employed a cross-sectional survey of on-ranch strategies and installed grazing exclosures in 24 irrigated pastures across three climatic regions of California. We monitored plant productivity and collected soil samples to analyze the response of microbial communities and soil properties (total C, N, etc.) to pasture rest from grazing along a gradient of irrigation management, soil fertility inputs, grazing management. This study aimed to evaluate how two years of grazing exclusion and pasture management practices interact to shape soil bacterial and fungal communities, with the expectation that increasing management effort would support more diverse and functionally beneficial microbial communities.

2. Methods

2.1. Irrigated pasture characteristics

This study includes 24 irrigated pastures spanning Northern California, covering three diverse climatic regions consisting of the lower elevation Sacramento Valley, the Sierra Nevada foothills, and intermountain regions of the Sierra Nevada and Southern Cascades (Fig. 1). The climate conditions across the sites include mild to cold wet winters and mild to hot dry summers. The pastures in this study occur across multiple landforms with varying degrees of land leveling, including laser-leveled low slope pastures in valleys, steep-sloped pasture in the foothills, and managed mountain meadows. All study pastures were grazed primarily by beef cattle, with some pastures grazed by sheep and cattle. The soils mapped at pasture sites ranged from clay to silt loams (Table 1; Natural Resources Conservation Service, 2022). We calculated a Management Effort (ME) score for each pasture based on livestock grazing, water, and nutrient management strategies. Each management variable was ranked from 1 to 3 (maximum ME score of 9), with a higher score indicating that higher levels of management efforts and intensification (e.g. practices that use more labor, inputs, or capital resources) were applied (Table 1). Grazing management was generally categorized based on livestock herd management: low-effort management with set stocking rates and continuous grazing throughout the year; moderate-effort management with inter-seasonal rotation and rest; and high-effort management with intra-seasonal pasture rotation and rest. Irrigation management effort and intensity was classified into three levels: low-effort systems using water diversions in wild-flooded pastures with minimal land leveling and water dispersion; moderate-effort systems

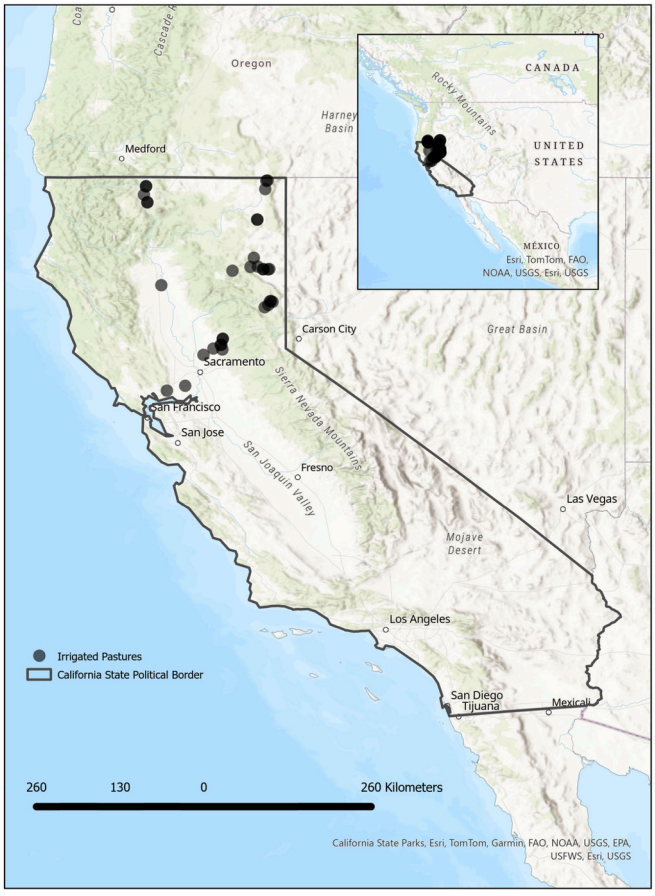


Fig. 1. Site map displaying the locations of the 24 irrigated pastures sampled in this study.

with some land leveling and gated flood pipes or risers and checks to control dispersion; and high-effort systems with controlled water distribution through center-pivot sprinkler systems or movable aboveground pod sprinkler systems (e.g. K-Line). Finally, soil nutrient management effort was categorized into three levels: low-effort with no organic or synthetic fertilizer inputs; moderate-effort with sporadic fertilizer amendments within the last five years, but not during the study; and high-effort with annual synthetic or organic fertilization inputs to pasture.

2.2. Soil sampling

We established three paired-plot monitoring sites across the soil moisture gradient representative of each irrigated pasture enrolled in the study. At each paired plot location, a 9 m^2 semi-permanent grazing exclosure was installed two years prior to soil microbial sampling. For each pasture, three intact 1″ by 12″ soil cores were collected from both inside and the outside of the exclosures at each paired plot, which were then pooled for soil chemical and physical analyses. This sampling depth coincides with the rooting depth of pasture forage plants that managers target for irrigation on these typically shallow soils. We sampled soil chemical traits associated with soil fertility during the early (April–May), middle (July–August), and end (September–October) of the growing season, and collected soil samples for microbial analyses during the end of the irrigation and growing season. To minimize contamination risks, a soil washing blank core was extracted and discarded at each sampling site prior to soil sample collection. Additionally, any residual soil was physically removed, and sampling equipment was sterilized with ethanol and dried between pastures.

2.3. Vegetation sampling

At each sampling location, we visually estimated species richness, percent grass species cover, and percent forb cover in three 0.09 M^2 frames within paired plots (i.e., inside and outside grazing exclosures). Values from the three frames were averaged to obtain a single estimate for each plot. We also harvested above ground biomass both inside and outside the exclosures at the end of each growing season (September–October). This allowed us to assess annual net primary production and calculate percent of herbaceous biomass utilized by livestock as a metric for grazing utilization.

2.4. Soil properties

Soil subsamples were first sieved to 2 mm and air dried until weights remained steady. Soil moisture was calculated by subtracting weight of soil after being oven-dried (at 105 °C for 24 h) from initial weight. For total organic carbon (TOC), we ground each sample to pass through a 0.25 mm sieve, and then treated samples with dilute acid to remove carbonate carbon. We then used a combustion instrument with an induction furnace coupled with a thermal conductivity detector and an IR detector system to measure combustion gases (Harris et al., 2001). For total nitrogen (N) and carbon (C), we used a similar approach as TOC, but without adding diluted acid to remove carbonates (AOAC Official Method 972.43, 1997). Subsamples of fresh soil were reserved to measure soil nitrate and ammonium via the flow injection analyzer method (Hofer, 2003; Keeney and Nelson, 1982; Knepel, 2003). Depending on pH, we measured extractable phosphorus as described by the Olsen and Bray methods (Olsen and Sommers, 1982; Prokopy, 1995). Exchangeable potassium was measured by ammonium acetate displacement and inductively coupled plasma atomic emission spectrometry (ICP-AES; Thomas, 1982).

2.5. Molecular analyses

DNA was extracted from soil samples with the DNeasy PowerLyzer Powersoil Kit following manufacturer protocols (QIAGEN, Hilden, Germany). Using PCR amplification to study bacteria and archaea, the V4 hypervariable region of 16S rRNA was sequenced using the 515-F (GTGCCAGCMGCCGCGGTAA) and 806-R (GGACTACHVGGGTWTCTAAT) primer pairs (Walters et al., 2016). For fungi, the first internal transcribed spacer (ITS1) region of the rRNA operon was sequenced using PCR amplification with the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) primer pairs (Walters et al., 2016). PCR was conducted in 40 μl triplicate reactions per sample, using 3 μl of extracted DNA, 3 μl of each primer, 20 μl of MyFi PCR Mix (Bioline, Taunton, MA, USA), and 11 μl of water. PCR consisted of an initial denaturing step at 95 °C for 1 min, 35 cycles of amplification (95 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s), and a final elongation step of 72 °C for 3 min. Illumina adapters and unique 12-bp error-correcting barcodes were also amplified using PCR to assist with demultiplexing. Each PCR process included negative controls without the DNA template to detect possible contamination. PCR products were cleaned using the Ultra Clean PCR Clean-Up Kit (MoBio Laboratories, Carlsbad, CA, USA), and quantified fluorescently using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA). All purified PCR products were pooled together in equimolar concentrations and sequenced on a 2 × 150 bp Illumina MiSeq platform at the Microbiome Core, University of Arizona.

2.6. Sequence processing

Amplicon sequences were demultiplexed using idemp (https://github.com/yhwu/idemp) and then processed using the DADA2 pipeline (Callahan et al., 2016) to filter, trim, and remove technical variants. Due to the varying length of the ITS region, cutadapt (Martin, 2011) was used

to remove ITS primer sequences. 16S sequences were trimmed to a uniform length of 140 bp. All sequence reads were filtered by quality including chimera removal, merging of paired-end reads, and the removal of sequences that contain more than two errors. Unique amplicon sequence variants (ASVs) were identified and dereplicated from the quality-filtered reads to represent distinct phylotypes. The taxonomic identity of each ASV was assigned using the DADA2-integrated Ribosomal Database Project (RDP) classifier (Wang et al., 2007) trained on the 16S rRNA SILVA database for bacteria and archaea (Quast et al., 2013) and the ITS UNITE database for fungi (Nilsson et al., 2019). Additional processing included the removal of ASVs with a domain assignment of chloroplast, mitochondria, or Eukaryota; ASVs that remained unclassified by the databases; and ASVs that were detected in negative control samples. We generated a total of 6,158,984 (mean per sample = 114,055 +/− 24,112) bacterial/archaeal sequences and 4,833,687 (mean per sample = 100,702 +/− 35,212) fungal sequences. Prokaryotic and fungal functional groups were inferred using FAPROTAX (Louca et al., 2016) and FUNGuild (Nguyen et al., 2016), respectively. Functional groups were manually filtered to include only confidence levels of "highly probable" or "probable".

2.7. Statistical analyses

All statistical analyses were performed in R version 4.0.3 (R Core Team, 2021). We rarefied the sequencing depth to 55,000 and 15,000 for 16S and ITS respectively. To determine the effect of grazing and pasture rest on microbial richness (number of different phylotypes) and Shannon diversity, paired *t*-tests were used. Pearson's correlation tests were applied to assess correlations between microbial richness and Shannon diversity. To assess impacts of soil properties, vegetation, and pasture management on microbial richness and Shannon diversity, linear mixed-effect models were used.

To examine the impacts of grazing and pasture management (ME Score) on microbial community composition, we calculated Euclidean distance or Bray-Curtis dissimilarity and employed permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). Differences in microbial community composition were then represented by non-metric multidimensional scaling (NMDS). We explored the associations among soil properties in ordination space using principal component analysis (PCA). Mantel tests were applied to examine the correlation between microbial community Bray-Curtis dissimilarity and the Euclidean distance matrix of soil variables, and also between the prokaryotic and fungal community compositions (Mantel, 1967). Multivariate statistics were performed using the vegan package (Oksanen et al., 2020). To measure differences in functional group abundance between grazed and rested sites, we applied paired Wilcoxon tests with false discovery rate (FDR) adjustment of 0.05.

3. Results

3.1. Vegetation and soil properties

We found grazing exclusion significantly influenced vegetative cover, with a higher grass cover in the rested plots (ANOVA, $F_{1,50} = 22.49$, $p \leq 0.001$; Supplementary Table 1). The percent of coverage by grasses and forbs was similar between pastures, but the vegetation species richness was significantly different based on pasture (ANOVA, adjusted $R^2 = 0.687$, $F_{27,24} = 5.15$, $p \leq 0.001$). Overall, soil properties significantly differed across pastures (PERMANOVA, $R^2 = 0.98$, $p \leq 0.001$) and differed to a lesser extent based on Management Effort (ME) score ($R^2 = 0.08$, $p = 0.022$; Supplementary Table 1).

3.2. Microbial community traits

The total number of unique amplicon sequence variants (ASVs) was 33,390 and 8573 for 16S and ITS, respectively. The average number of ASVs per soil sample was 2011 (+/− 402) for 16S and 509 (+/− 171) for ITS. Microbial richness was not significantly different between grazed and rested plots (paired *t*-test, $t_{22} = -0.82$, $p = 0.421$ for 16S; $t_{16} = -0.36$, $p = 0.727$ for ITS; Fig. 2A, B). Bacterial/Archaeal (16S) and fungal (ITS) richness were not correlated (Pearson's $r = 0.10$, $p = 0.452$). Shannon diversity was also not significantly different between grazed and rested plots (paired t-test, $t_{22} = -1.53$, $p = 0.141$ for 16S; $t_{16} = -0.65$, $p = 0.526$ for ITS; Supplementary Fig. 2). Bacterial/Archaeal (16S) and fungal (ITS) Shannon diversity were not correlated (Pearson's $r = -0.04$, $p = 0.754$). ME score did not significantly impact microbial richness (ANOVA, marginal $r^2 = 0.063$, $p = 0.088$ for 16S; marginal $r^2 = 0.003$, conditional $r^2 = 0.205$, $p = 0.788$ for ITS; Supplementary Table 2) or Shannon diversity (marginal $r^2 = 0.083$, conditional $r^2 = 0.206$, $p = 0.073$ for 16S; marginal $r^2 = 0.008$, $p = 0.612$ for ITS). Similarly, the individual management variables (irrigation, fertilization, and herd management) did not significantly impact microbial richness or Shannon diversity (Supplementary Table 2). Bacterial/Archaeal and fungal richness and bacterial/archaeal Shannon diversity were not significantly different based on soil or vegetation properties (Supplementary Table 2). Fungal Shannon diversity was significantly impacted by total nitrogen (TN; marginal $r^2 = 0.275$, $p = 0.044$). (See Supplementary Fig. 2.)

The soil bacterial and archaeal communities mainly consisted of Alphaproteobacteria (13.4 %), Thermoliophilia (11.1 %), Gammaproteobacteria (9.9 %), and Actinobacteria (8.2 %; Supplementary Fig. 3 A). The soil fungal communities mainly consisted of Sodariomycetes (32.9 %), Dothideomycetes (20.5 %), Mortierellomycetes (9.4 %), and Agaricomycetes (6.8 %; Supplementary Fig. 3B). Bacterial/archaeal and fungal community similarity patterns were both correlated (Mantel test, $r = 0.56$, $p \leq 0.001$), and were also correlated with soil properties (Mantel test, $r = 0.31$, $p \leq 0.001$ for 16S; $r = 0.51$, $p \leq 0.001$ for ITS). Microbial community composition did not differ based on grazing rest (PERMANOVA, $R^2 = 0.02$, $p = 0.747$ for 16S; $R^2 = 0.02$, $p = 0.999$ for ITS; Fig. 2C, D). Community similarity patterns were significantly different based on pasture for both bacterial/archaeal (PERMANOVA, $R^2 = 0.78$, $p \leq 0.001$; Fig. 2C) and fungal ($R^2 = 0.71$, $p \leq 0.001$; Fig. 2D) communities. ME score was not associated with bacterial/archaeal community similarity patterns (PERMANOVA, $R^2 = 0.03$, $p = 0.208$ for 16S), but was very weakly associated with fungal community similarity patterns ($R^2 = 0.04$, $p = 0.024$ for ITS).

Within the bacterial/archaeal communities, there were nearly significant differences in the abundance of putative fermenting bacteria (mainly *Opitutaceae* and *Spirochaetaceae*) and nitrifying bacteria (mainly *Nitrosomonadaceae* and *Nitrososphaeraceae*) between grazed and rested plots (Supplementary Table 3). While these functional groups were not significant after FDR correction, there was a tendency of more fermenters in the grazed plots and more nitrifiers in the rested plots (Fig. 3A, B). Within the fungal communities, there were nearly significant differences in the abundances of putative plant pathogens and arbuscular mycorrhizal fungi (AMF) between the grazed and rested plots before FDR correction (Supplementary Table 3). Both plant pathogens (mainly *Entorrhiza*) and AMF (*Glomus*) were more abundant in the rested exclosure plots (Fig. 3C, D), though, in more conservative interpretation, the results were not significant after FDR correction of 0.05.

4. Discussion

Soil microbial communities are shaped by a diversity of abiotic and biotic processes ranging from soil formation factors (e.g. climate, age, parent material, organisms, etc.) to land use and management factors such as soil plant nutrient inputs, livestock grazing, and irrigation. The effects of livestock grazing extend into belowground ecosystems via nutrient and organic matter inputs (Bardgett and Wardle, 2003), and physical soil disturbances (Bilotta et al., 2007). In grassland ecosystems, the quantity, diversity, and composition of soil bacterial and fungal communities can shift in response to livestock grazing and can

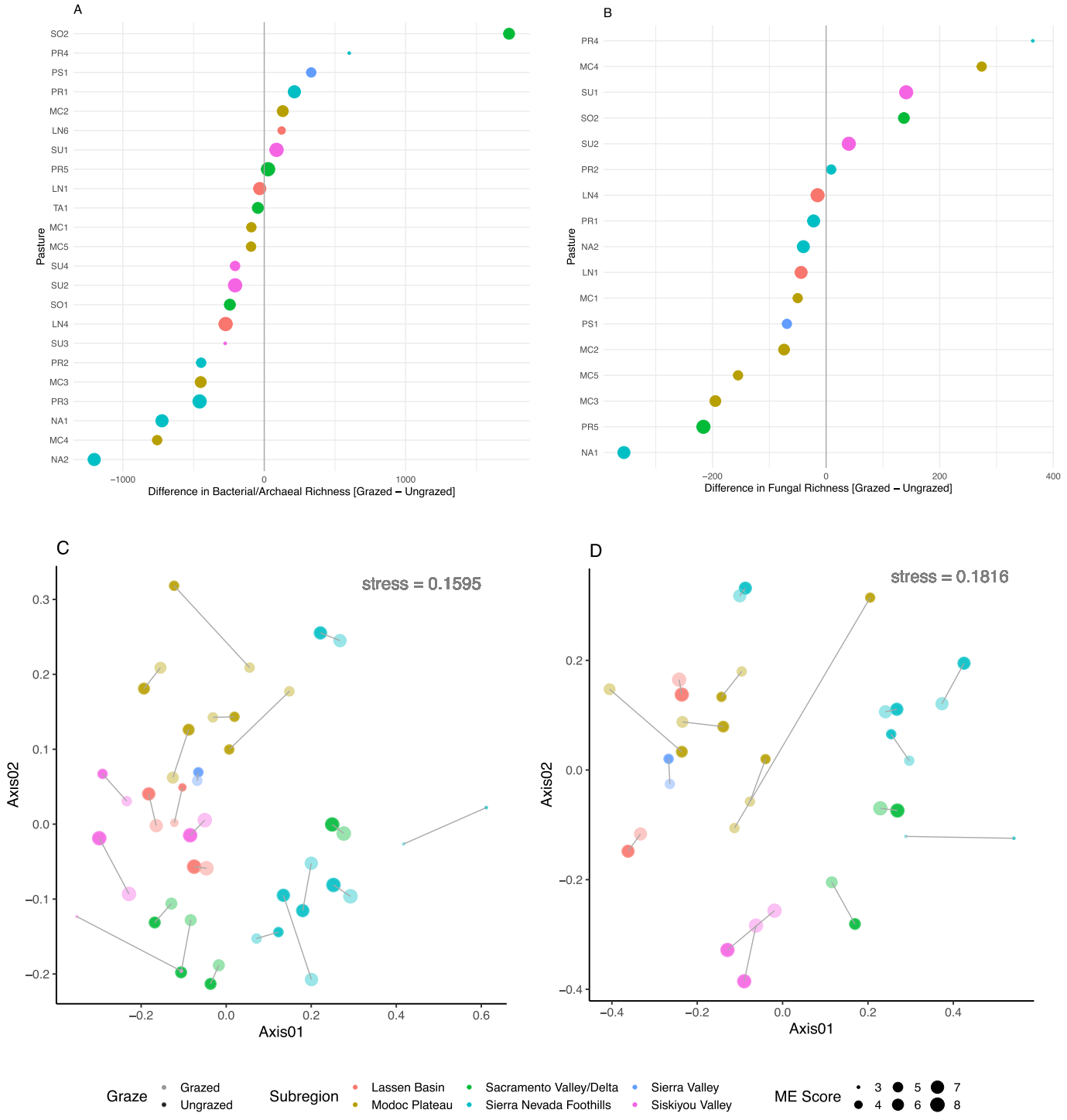


Fig. 2. Soil microbial richness and community composition. Differences in (A) bacterial/archaeal and (B) fungal richness between grazed and ungrazed plots. Positive and negative values indicate higher richness in grazed and ungrazed plots, respectively. Differences in (C) bacterial/archaeal and (D) fungal community composition calculated by Bray-Curtis dissimilarity and represented by non-metric multidimensional scaling (NMDS). Lines connect grazed and ungrazed plots within the same pastures.

additionally fluctuate based on grazing intensity (Xun et al., 2018; Gou et al., 2015). Here, we analyzed the soil chemistry, vegetation, and microbial communities of Californian irrigated pastures using a paired assessment of grazed and grazing-rested plots across gradients of effort to manage soil nutrients, irrigation water, and livestock grazing. We found that two years of rest from grazing did not significantly affect soil microbial diversity or composition (Fig. 2), highlighting a potential resilience of soil microbial communities to short term changes in grazing management. Understanding the impacts of long-term management changes on soil microbial communities and the ecosystem services they support is essential to furthering the sustainability of these important agroecosystems that are most commonly used as summer grazing resources.

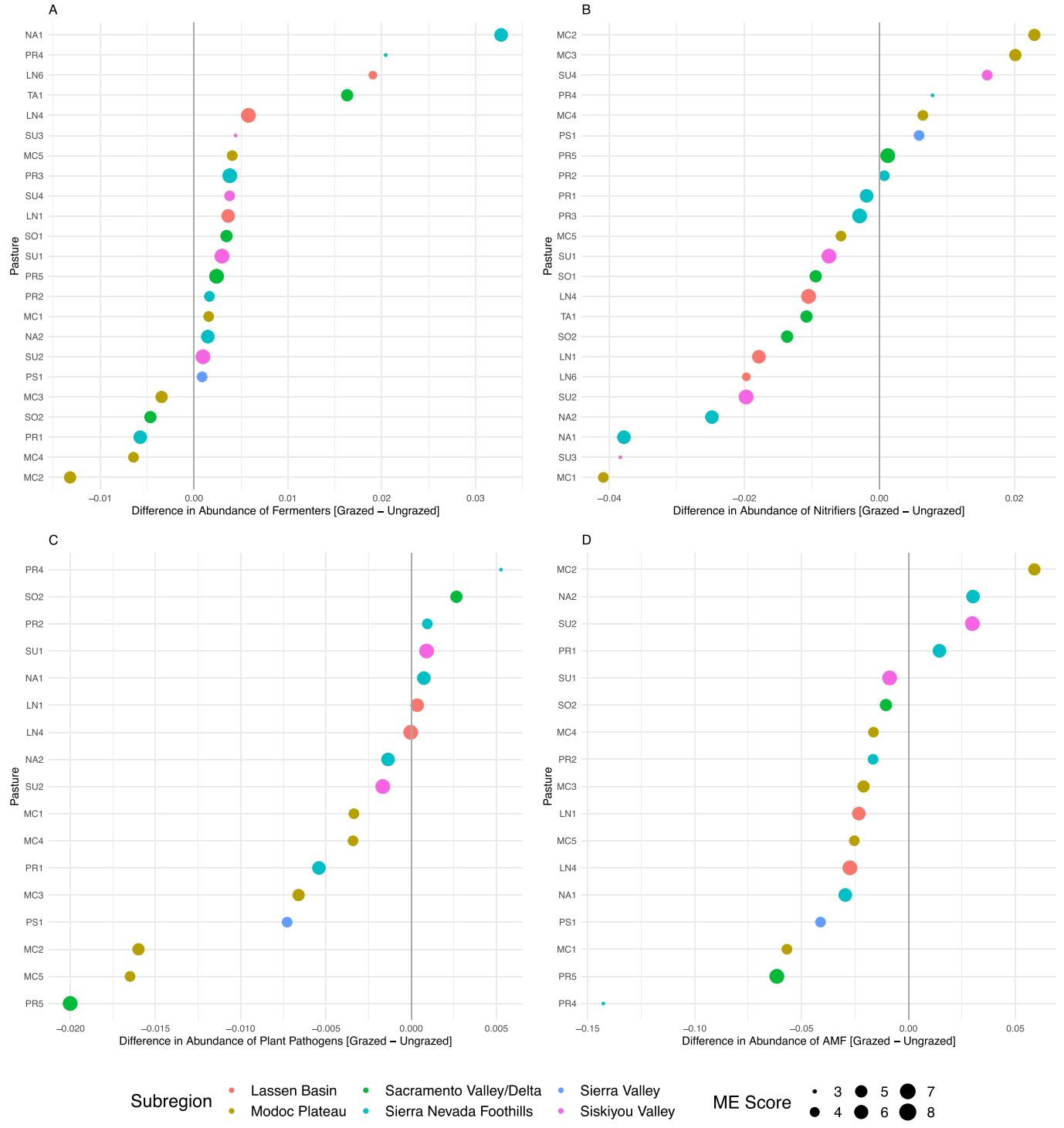


Fig. 3. Functional group abundance. Differences in the abundance of (A) fermenters, (B) nitrifiers, (C) plant pathogens, and (D) arbuscular mycorrhizal fungi (AMF) between grazed and ungrazed plots. Positive and negative values indicate higher abundance in grazed and ungrazed plots, respectively.

4.1. Pasture-specific vegetation and soil properties affect microbial communities

This study spanned a diverse range of irrigated pastures in Northern California, including three climatic regions with contrasting landforms, soil types, and management practices. We identified strong pasture-specific effects on soil chemical properties and soil microbial communities (Fig. 2C, D; Supplementary Table 1). Microbial diversity and composition can be strongly influenced by soil characteristics (Robson and Abbott, 1989; Dahlgren et al., 1997; Li et al., 2016; Carey et al., 2020), which vary among different parent materials, geomorphologies, and climatic conditions (George et al., 2020; Ford et al., 2013). For example, in a study that analyzed bacterial and fungal communities across numerous climates and biomes, soil C content and pH were key drivers of microbial community composition (Bastida et al., 2021). As such, we observed significant differences in bacterial/archaeal and fungal community similarities based on pasture (Fig. 2C, D) and identified a correlation between soil properties and microbial community

composition (Supplementary Fig. 1), suggesting that the inherent variation in soil properties across pastures and biomes is a dominant driver of microbial variability (Almela et al., 2021; Trivedi et al., 2016). Additionally, we identified a relationship between fungal Shannon diversity and TN (Supplementary Table 2), which exemplifies the need for further research to define the relationship between soil chemistry and microbial diversity. Similar studies have identified stronger associations between microbial communities, environmental variables, and pasture management, rather than exclusively to livestock grazing (Yang et al., 2013; Ford et al., 2013; Acharya et al., 2021; Yang et al., 2019a, 2019b; Cleavenger et al., 2023; Farrell et al., 2020).

4.2. Variable responses of microbial functional groups to grazing exclusion

From a functional perspective, prior studies found that grazing can affect the abundance of soil microbes involved in N cycling (Patra et al., 2005; Tracy and Frank, 1998; T. Liu et al., 2011). In particular, higher grazing intensity has been linked with higher nitrification and denitrification activities (Le Roux et al., 2003; Taddese et al., 2007). These changes are hypothesized to be the result of grazing factors such as inputs from livestock waste, reduced plant litter from defoliation, and higher cover of legumes due to reduced canopy height (Le Roux et al., 2003; Patra et al., 2005). Within our study, there was an apparent, but nonsignificant trend with more nitrifiers in the rested plots (Fig. 3B). This is inconsistent with previous findings in which nitrifying bacteria and archaea are instead more abundant in grazed plots (Le Roux et al., 2008; Patra et al., 2005). While the duration of grazing exclusion may have been too brief to observe a significant difference in this study, the lack of nitrifiers in grazed plots may also be explained by a corresponding decline in fertility, specifically NH_4^+ content, often found in heavily grazed grasslands (Pan et al., 2018). In cases where livestock grazing results in the degradation of soil physiochemical traits (NH_4^+, SO_3^-, total N, soil moisture; Zhong et al., 2014), nitrifying prokaryotes and their resulting nitrification rates are reported to be negatively impacted as well (Wang et al., 2022; Pan et al., 2018). Californian irrigated pastures are highly productive and are often annually harvested by livestock at high rates of total aboveground biomass utilization, which over time could lead to soil physiochemical deficiencies. More investigation is needed, perhaps with a longer duration of grazing exclusion and a gradient of grazing intensity, to confirm a significant trend in which nitrifiers are less abundant in grazed plots due to reduced soil fertility.

Our observation of a higher abundance of fermenting bacteria within grazed sites (Fig. 3A) aligns with the ecological role of these bacteria, which are key components of cow rumen and dung microbiota (Ozbayram et al., 2018). Common classes of fermenting bacteria that are involved in the breakdown of forage in ruminant digestive systems are Fibrobacterota, Bacteroidetes, and Firmicutes (Lwin et al., 2012), many of which have been found in cow dung (Christy et al., 2014; Girija et al., 2013). This suggests that the dispersal or preferential recruitment of these microorganisms may be facilitated by the deposition of animal waste. *Escherichia coli* associated with cattle feces deposition is largely attenuated within 1 m of vegetated buffers (Tate et al., 2006; Atwill et al., 2002). However, the degradation of starches via fermentation commonly occurs in soils without grazing inputs (Dadwal et al., 2019) and the contribution of rumen fermenters to the soil microbiome has not been well quantified. To fully identify a significant impact of livestock on the abundance of soil fermenters, more focus should be directed towards the microbial composition and fate and transport of livestock dung-borne microbes in pasture soils.

Prior studies have found that grazing can facilitate both an increase (Wearn and Gange, 2007) and decrease (Barber et al., 2012; Yang et al., 2020) in AMF colonization. The sensitivity of AMF to disturbance can vary based on host species, fungal community composition, and type of disturbance (Hart and Reader, 2004; Van der Heyde et al., 2017). By nature of their relationship with plant hosts, AMF are sensitive to the uproot and removal of plants by heavy livestock grazing (Yang et al., 2020). Our observations of higher abundances of AMF in our rested sites (Fig. 3D), although nonsignificant after adjusting for false detection error, suggest a negative correlation between grazing and AM fungi. This, combined with our observations of significantly reduced grass cover in grazed plots (Supplementary Table 1), suggests that the physical disturbance and altering of plant community dynamics by livestock leads to the reduction of AMF within soil microbial communities. An increased duration of grazing exclusion may be necessary to confirm a positive effect of grazing rest on AMF abundance.

4.3. Long- and short-term effects of grazing management on pasture vegetation

As we observed an effect of grazing on grass cover (Supplementary Table 1), but not on soil microbial diversity or soil community composition (Fig. 2), it is possible that the duration and scale of grazing removal in this study was insufficient and did not significantly impact the belowground ecosystem, thus leading to non-significant differences based on grazing alone. In this case, the deep-rooted, perennial grasses receive ample moisture from irrigation (Neal et al., 2012) and may have had high enough regrowth rates to negate any effects of defoliation that would be seen on rangeland grazing sites that are subject to drought and subsequently less regrowth. It is also possible that the extent of legacy management factors such as nutrients, water, and livestock rotation and stocking rates may have been dominant and indirectly affected microbial communities via their influence on soil properties (Wang et al., 2021; Zhang et al., 2023). The management practices employed in this study had been implemented for more than five years, and changes in grazing management may require more time to produce quantifiable differences in soil microbial communities and other soil health indicators. Further research regarding the effects of specific pasture management techniques on soil properties is needed to elucidate soil-mediated effects of grazing on microbial communities.

5. Conclusion

Our study used 16S rRNA and ITS amplicon analyses to assess the effect of two years of grazing exclusion on soil microbial communities in Californian irrigated pastures. Microbial diversity and community composition were unaffected by grazing rest and slight trends were present among putative microbial functional groups. Microbial community patterns were correlated with soil properties and strongly associated with individual pastures. When utilizing microbial community to inform soil conservation, assessments should consider the potential of the site. Thus, grazing land restoration efforts should note that variation in soil properties and microbial communities were mostly attributed to inherent spatial differences between sites possibly linked to variation in landscape position, microclimates, parent material, soil texture, soil weathering, dry atmospheric nutrient deposition, and uncertain legacy effects from past land management. Grazing exclusion as employed in this experiment did not significantly influence soil microbial communities, and a longer duration or larger exclusion area may be necessary to observe differences in an experimental (non-production) setting. Microbial diversity metrics are beneficial tools to inform our understanding of the health of soils and the dependent supply of ecosystem services, but their limitations should be recognized and mitigated with additional soil health indicators (i.e., soil physiochemical data; Fierer et al., 2021). Furthermore, this study highlights the need to detangle the role mycorrhizal and pathogenic fungi may have in pasture productivity and whether large scale fungal inoculation can enhance nutrient uptake and yields as found in other grass cropping systems like maize (Lutz et al., 2023). This research is among the first of its kind in California's unique irrigated pasture systems and offers insights into potential connections between management practices and the soil microbiome in this forage

production system. A better understanding of microbial responses to changes in livestock grazing management can accelerate and optimize resources for attaining land management goals.

CRediT authorship contribution statement

Madison L. Morris: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Danny J. Eastburn:** Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Leslie M. Roche:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Josh Davy:** Writing – review & editing, Investigation, Data curation. **Morgan Doran:** Writing – review & editing, Investigation, Data curation. **Betsy Karle:** Writing – review & editing, Investigation, Data curation. **David Lile:** Writing – review & editing, Investigation, Data curation. **Tracy Schohr:** Writing – review & editing, Investigation, Data curation. **Laura Snell:** Writing – review & editing, Investigation, Data curation. **Dan Macon:** Writing – review & editing, Investigation, Data curation. **Grace Woodmansee:** Writing – review & editing, Investigation, Data curation. **María Touceda-Suárez:** Writing – review & editing, Formal analysis, Data curation. **Albert Barberán:** Writing – review & editing, Validation, Resources, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by a California Department of Water Resources Agreement (#4600012155) and the USDA Western Sustainable Agriculture Research and Education Program (OW16–013).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Leslie Roche reports was provided by California Department of Water Resources. Leslie Roche reports was provided by Western Sustainable Agriculture Research & Education. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106419.

Data availability

Data are publicly available on Figshare: https://doi.org/10.6084/m9.figshare.29275181.v1

References

Abril, A., Bucher, E.H., 1999. The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. Appl. Soil Ecol. 12 (2), 159–167.
Acharya, M., Ashworth, A.J., Yang, Y., Burke, J.M., Lee, J.A., Acharya, R.S., 2021. Soil microbial diversity in organic and non-organic pasture systems. PeerJ 9, e11184.
Almela, P., Justel, A., Quesada, A., 2021. Heterogeneity of microbial communities in soils from the Antarctic peninsula region. Front. Microbiol. 12, 280.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26 (1), 32–46.
AOAC Official Method 972.43, 1997. Microchemical Determination of Carbon, Hydrogen, and Nitrogen, Automated Method. In: Cunniff, P. (Ed.), Official Methods of Analysis of AOAC International, 16th ed. AOAC International, essay, pp. 5–6.
Atwill, E.R., Hou, L., Karle, B.M., Harter, T., Tate, K.W., Dahlgren, R.A., 2002. Transport of *Cryptosporidium parvum* Oocysts through Vegetated Buffer Strips and Estimated Filtration Efficiency. Appl. Environ. Microbiol. 68 (11), 5517–5527.
Barber, N.A., Adler, L.S., Theis, N., Hazzard, R.V., Kiers, E.T., 2012. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. Ecology 93 (7), 1560–1570.
Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84 (9), 2258–2268.
Bastida, F., Eldridge, D.J., García, C., Kenny Png, G., Bardgett, R.D., Delgado-Baquerizo, M., 2021. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. The ISME Journal 2021 15:7 15 (7), 2081–2091.
Bilotta, G.S., Brazier, R.E., Haygarth, P.M., 2007. The impacts of grazing animals on the quality of soils, vegetation, and surface waters in intensively managed grasslands. Adv. Agron. 94, 237–280.
Byrnes, R.C., Eastburn, D.J., Tate, K.W., Roche, L.M., 2018. A Global Meta-Analysis of Grazing Impacts on Soil Health Indicators. J. Environ. Qual. 47 (4), 758–765.
California Department of Food and Agriculture. (n.d.). California Agricultural Statistics Review 2019–2020.
California Department of Forestry and Fire Protection, 2018. California's Forests and Rangelands 2017 Assessment.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583.
Carey, C.J., Weverka, J., DiGaudio, R., Gardali, T., Porzig, E.L., 2020. Exploring variability in rangeland soil organic carbon stocks across California (USA) using a voluntary monitoring network. Geoderma Regional 22, e00304.
Christy, P.M., Gopinath, L.R., Divya, D., 2014. Microbial dynamics during anaerobic digestion of cow dung. International Journal of Plant, Animal and Environmental Sciences 4 (4), 86–94.
Cleavenger, C., Chen, Y., Barberán, A., 2023. Mesquite-associated soil and phyllosphere microbial communities differ across land-use types in drylands. Elementa: Science of the Anthropocene 11 (1), 00026.
Couvreur, M., Christiaen, B., Verheyen, K., Hermy, M., 2004. Large herbivores as mobile links between isolated nature reserves through adhesive seed dispersal. Appl. Veg. Sci. 7 (2), 229–236.
Dadwal, A., Sharma, S., & Satyanarayana, T. (2019). Diversity in cellulose-degrading microbes and their cellulases: Role in ecosystem sustainability and potential applications. *Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Volume 2. Soil & Agroecosystems*, 375–402.
Dahlgren, R.A., Boettinger, J.L., Huntington, G.L., Amundson, R.G., 1997. Soil development along an elevational transect in the western Sierra Nevada. California. Geoderma 78 (3–4), 207–236.
Davy, J.S., Roche, L.M., Robertson, A.V., Nay, D.E., Tate, K.W., 2015. Introducing cattle grazing to a noxious weed-dominated rangeland shifts plant communities. Calif. Agric. 69 (4), 230–236.
Demeter, L., Molnár, Á.P., Bede-Fazekas, Á., Öllerer, K., Varga, A., Szabados, K., Tucakov, M., Kiš, A., Biró, M., Marinkov, J., Molnár, Z., 2021. Controlling invasive alien shrub species, enhancing biodiversity and mitigating flood risk: A win–win–win situation in grazed floodplain plantations. J. Environ. Manag. 295, 113053.
Derner, J.D., Smart, A.J., Toombs, T.P., Larsen, D., McCulley, R.L., Goodwin, J., Sims, S., Roche, L.M., 2018. Soil Health as a Transformational Change Agent for US Grazing Lands Management. Rangel. Ecol. Manag. 71 (4), 403–408.
Dubeux, J.C.B., Sollenberger, L.E., Mathews, B.W., Scholberg, J.M., Santos, H.Q., 2007. Nutrient cycling in warm-climate grasslands. Crop Sci. 47 (3), 915–928.
Farrell, H.L., Barberán, A., Danielson, R.E., Fehmi, J.S., Gornish, E.S., 2020. Disturbance is more important than seeding or grazing in determining soil microbial communities in a semiarid grassland. Restor. Ecol. 28 (S4).
Fierer, N., Wood, S.A., Bueno de Mesquita, C.P., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol. Biochem. 153, 108111.
Ford, H., Rousk, J., Garbutt, A., Jones, L., Jones, D.L., 2013. Grazing effects on microbial community composition, growth and nutrient cycling in salt marsh and sand dune grasslands. Biol. Fertil. Soils 49 (1), 89–98.
George, M.R., Roche, L.M., Eastburn, D.J., 2020. Vegetation change and ecosystem services. University of California, Agriculture and Natural Resources, In Vegetation Change and Ecosystem Services.
Girija, D., Deepa, K., Xavier, F., Antony, I., Shidhi, P.R., 2013. Analysis of cow dung microbiota—A metagenomic approach. NISCAIR-CSIR 12 (3), 372–378.
Gou, Y., Nan, Z., Hou, F., 2015. Diversity and structure of a bacterial community in grassland soils disturbed by sheep grazing, in the Loess Plateau of northwestern China. Genet. Mol. Res. 14 (4), 16987–16999.
Graham, S.L., Laubach, J., Hunt, J.E., Mudge, P.L., Nuñez, J., Rogers, G.N.D., Buxton, R. P., Carrick, S., Whitehead, D., 2022. Irrigation and grazing management affect leaching losses and soil nitrogen balance of lucerne. Agric. Water Manag. 259, 107233.
Harris, D., Horwáth, W.R., Van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13 isotopic analysis. Soil Sci. Soc. Am. J. 65, 1853–1856.
Hart, M.M., Reader, R.J., 2004. Do arbuscular mycorrhizal fungi recover from disturbance differently? International Society for Tropical Ecology 45 (1), 97–111.
Hofer, S., 2003. Determination of Ammonia (Salicylate) in 2M KCl soil extracts by Flow Injection Analysis. QuikChem Method 12–107–06-2-A. Lachat Instruments, Loveland, CO.
Holland, E.A., Parton, W.J., Detling, J.K., Coppock, D.L., 1992. Physiological responses of plant populations to herbivory and their consequences for ecosystem nutrient flow. Am. Nat. 140 (4), 685–706.
Huntsinger, L., Hruska, T.V., Oviedo, J.L., Shapero, M.W.K., Nader, G.A., Ingram, R.S., Beissinger, S.R., 2017. Save water or save wildlife? Water use and conservation in the central Sierran foothill oak woodlands of California, USA. Ecol. Soc. 22 (2).

Keeney, D. R. and Nelson, D. W. (1982). Nitrogen-inorganic forms. Pp. 643–698. In: A. L. Page (ed.) Methods of soil analysis: Part 2. Chemical and microbiological properties. ASA Monograph Number 9.

Knepel, K. (2003). Determination of Nitrate in 2M KCl soil extracts by Flow Injection Analysis.QuikChem Method 12–107–04-1-B. Lachat Instruments, Loveland, CO.

Lambie, S.M., Mudge, P.L., Stevenson, B.A., 2021. Microbial community composition and activity in paired irrigated and non-irrigated pastures in New Zealand. Soil Research 60 (4), 337–348.

Le Roux, X., Bardy, M., Loiseau, P., Louault, F., 2003. Stimulation of soil nitrification and denitrification by grazing in grasslands: Do changes in plant species composition matter? Oecologia 137 (3), 417–425.

Le Roux, X., Poly, F., Currey, P., Commeaux, C., Hai, B., Nicol, G.W., Prosser, J.I., Schloter, M., Attard, E., Klumpp, K., 2008. Effects of aboveground grazing on coupling among nitrifier activity, abundance and community structure. The ISME Journal 2008 2 (2), 221–232.

Li, Y., Lin, Q., Wang, S., Li, X., Liu, W., Luo, C., Zhang, Z., Zhu, X., Jiang, L., Li, X., 2016. Soil bacterial community responses to warming and grazing in a Tibetan alpine meadow. FEMS Microbiol. Ecol. 92 (1).

Liu, T., Nan, Z., Hou, F., 2011. Culturable autotrophic ammonia-oxidizing bacteria population and nitrification potential in a sheep grazing intensity gradient in a grassland on the loess plateau of Northwest China. Can. J. Soil Sci. 91 (6), 925–934.

Liu, J., Zhang, Q., Li, Y., Di, H., Xu, J., Li, J., Guan, X., Xu, X., Pan, H., 2016. Effects of pasture management on soil fertility and microbial communities in the semi-arid grasslands of Inner Mongolia. J. Soils Sediments 16 (1), 235–242.

Louca, S., Jacques, S.M.S., Pires, A.P.F., Leal, J.S., Srivastava, D.S., Parfrey, L.W., Farjalla, V.F., Doebeli, M., 2016. High taxonomic variability despite stable functional structure across microbial communities. Nature Ecology & Evolution 1 (1), 1–12.

Lutz, S., Bodenhausen, N., Hess, J., Valzano-Held, A., Waelchli, J., Deslandes-Hérold, G., Schlaeppi, K., Van Der Heijden, M.G.A., 2023. Soil microbiome indicators can predict crop growth response to large-scale inoculation with arbuscular mycorrhizal fungi. Nat. Microbiol. 8 (12), 2277–2289.

Lwin, K.O., Kondo, M., Ban-Tokuda, T., Lapitan, R.M., Del-Barrio, A.N., Fujihara, T., Matsui, H., 2012. Ruminal fermentation and microbial ecology of buffaloes and cattle fed the same diet. Anim. Sci. J. 83 (12), 767–776.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27 (2), 209–220.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal 17 (1), 10–12.

Natural Resources Conservation Service, United States Department of Agriculture, 2022. Web Soil Survey. Accessed 04/24/22.

Neal, J.S., Murphy, S.R., Harden, S., Fulkerson, W.J., 2012. Differences in soil water content between perennial and annual forages and crops grown under deficit irrigation and used by the dairy industry. Field Crop Res. 137, 148–162.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.

Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47 (D1), D259–D264.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. Vegan: community ecology package. R package version 2, 5–7.

Olsen, S. R. & Sommers, L. E. (1982). Phosphorus. Pp. 403–430. In A. L. Page, et al. (eds.) Methods of soil analysis: Part 2. Chemical and microbiological properties. Agron. Mongr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Ozbayram, E., Ince, O., Ince, B., Harms, H., Kleinsteuber, S., 2018. Comparison of Rumen and Manure Microbiomes and Implications for the Inoculation of Anaerobic Digesters. Microorganisms 6 (1), 15.

Pan, H., Liu, H., Liu, Y., Zhang, Q., Luo, Y., Liu, X., Liu, Y., Xu, J., Di, H., Li, Y., 2018. Understanding the relationships between grazing intensity and the distribution of nitrifying communities in grassland soils. Sci. Total Environ. 634, 1157–1164.

Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J.I., Richaume, A., Le Roux, X., 2005. Effects of grazing on microbial functional groups involved in soil dynamics. Ecol. Monogr. 75 (1), 65–80.

Plue, J., Cousins, S.A.O., 2018. Seed dispersal in both space and time is necessary for plant diversity maintenance in fragmented landscapes. Oikos 127 (6), 780–791.

Porensky, L.M., McGee, R., Pellatz, D.W., 2020. Long-term grazing removal increased invasion and reduced native plant abundance and diversity in a sagebrush grassland. Global Ecology and Conservation 24, e01267.

Prokopy, W.R., 1995. Phosphorus in 0.5 M sodium bicarbonate soil extracts. QuikChem Method 12–115–01-1-B. Lachat Instruments, Milwaukee, WI.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596.

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rhodes, A.C., Rutledge, J., DuPont, B., Plowes, R.M., Gilbert, L.E., 2021. Targeted grazing of an invasive grass improves outcomes for native plant communities and wildlife habitat. Rangel. Ecol. Manag. 75, 41–50.

Robson, A. D., & Abbott, L. K. (1989). The effect of soil acidity on microbial activity in soils. In: A. D. Robson (Ed.), *Soil Acidity and Plant Growth,* 139–166.

Roche, L., 2016. Adaptive Rangeland Decision-Making and Coping with Drought. Sustainability 8 (12), 1334.

Roche, L.M., Cutts, B.B., Derner, J.D., Lubell, M.N., Tate, K.W., 2015a. On-Ranch Grazing Strategies: Context for the Rotational Grazing Dilemma. Rangel. Ecol. Manag. 68 (3), 248–256.

Roche, L.M., Schohr, T.K., Derner, J.D., Lubell, M.N., Cutts, B.B., Kachergis, E., Eviner, V. T., Tate, K.W., 2015b. Sustaining Working Rangelands: Insights from Rancher Decision Making. Rangel. Ecol. Manag. 68 (5), 383–389.

Rusch, G.M., Oesterheld, M., Oesterheld, M., 1997. Relationship between productivity, and species and functional group diversity in grazed and non-grazed pampas grassland. Oikos 78 (3), 519.

Schönbach, P., Wan, H., Gierus, M., Bai, Y., Müller, K., Lin, L., Susenbeth, A., Taube, F., 2011. Grassland responses to grazing: Effects of grazing intensity and management system in an Inner Mongolian steppe ecosystem. Plant Soil 340 (1), 103–115.

Shao, C., Chen, J., Li, L., 2013. Grazing alters the biophysical regulation of carbon fluxes in a desert steppe. Environ. Res. Lett. 8 (2), 025012.

Shapero, M., Dronova, I., Macaulay, L., 2017. Implications of changing spatial dynamics of irrigated pasture, California's third largest agricultural water use. Sci. Total Environ. 605–606, 445–453.

Steinfeld, H., Wassenaar, T., 2007. The role of livestock production in carbon and nitrogen cycles. Annu. Rev. Environ. Resour. 32, 271–294.

Taddese, G., Peden, D., Hailemariam, A., Jobre, Y., Ayalneh, W., 2007. Effect of livestock grazing on soil micro-organisms of cracking and self mulching vertisol. Ethiopian Veterinary Journal 141–150.

Tate, K.W., Atwill, E.R., Bartolome, J.W., Nader, G., 2006. Significant *Escherichia coli* Attenuation by Vegetative Buffers on Annual Grasslands. J. Environ. Qual. 35 (3), 795–805.

Thomas, G. W. (1982). Exchangeable cations. pp 159–165. In: A.L. Page et al. (ed.) Methods of soil analysis: Part 2. Chemical and microbiological properties. ASA Monograph Number 9.

Touhami, D., Condron, L.M., McDowell, R.W., Moss, R., 2023. Effects of long-term phosphorus fertilizer inputs and seasonal conditions on organic soil phosphorus cycling under grazed pasture. Soil Use Manag. 39 (1), 385–401.

Tracy, B.F., Frank, D.A., 1998. Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park. Oecologia 1998 114:4 114 (4), 556–562.

Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C., Singh, B.K., 2016. Response of soil properties and microbial communities to agriculture: Implications for primary productivity and soil health indicators. Front. Plant Sci. 7 (990), 1–13.

Van der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11 (3), 296–310.

Van der Heyde, M., Ohsowski, B., Abbott, L.K., Hart, M., 2017. Arbuscular mycorrhizal fungus responses to disturbance are context-dependent. Mycorrhiza 27 (5), 431–440.

Wakelin, S.A., Gregg, A.L., Simpson, R.J., Li, G.D., Riley, I.T., McKay, A.C., 2009. Pasture management clearly affects soil microbial community structure and N-cycling bacteria. Pedobiologia 52 (4), 237–251.

Wakelin, S., Mander, C., Gerard, E., Jansa, J., Erb, A., Young, S., Condron, L., O'Callaghan, M., 2012. Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. Appl. Soil Ecol. 61, 40–48.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2016. Improved Bacterial 16S rRNA Gene (V4 and V4–5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. MSystems 1 (1), e00009–e00015.

Wang, B., Wu, Y., Chen, D., Hu, S., Bai, Y., 2021. Legacy effect of grazing intensity mediates the bottom-up controls of resource addition on soil food webs. J. Appl. Ecol. 58 (5), 976–987. https://doi.org/10.1111/1365-2664.13825.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73 (16), 5261–5267.

Wang, X., Wang, C., Zhou, C., Zuo, S., Ji, Y., Lamao, Q., & Huang, D. (2022). Grazing horse effects on desert grassland soil gross nitrification and denitrification rates in northern China. *Agriculture 2022, 12*(7), 1036.

Wearn, J.A., Gange, A.C., 2007. Above-ground herbivory causes rapid and sustained changes in mycorrhizal colonization of grasses. Oecologia 153 (4), 959–971.

Xun, W., Yan, R., Ren, Y., Jin, D., Xiong, W., Zhang, G., Cui, Z., Xin, X., Zhang, R., 2018. Grazing-induced microbiome alterations drive soil organic carbon turnover and productivity in meadow steppe. Microbiome 6 (1), 1–13.

Yang, F., Niu, K., Collins, C.G., Yan, X., Ji, Y., Ling, N., Zhou, X., Du, G., Guo, H., Hu, S., 2019a. Grazing practices affect the soil microbial community composition in a Tibetan alpine meadow. Land Degrad. Dev. 30 (1), 49–59.

Yang, X., Chen, J., Shen, Y., Dong, F., Chen, J., 2020. Global negative effects of livestock grazing on arbuscular mycorrhizas: A meta-analysis. Sci. Total Environ. 708, 134553.

Yang, Y., Wu, L., Lin, Q., Yuan, M., Xu, D., Yu, H., Hu, Y., Duan, J., Li, X., He, Z., Xue, K., van Nostrand, J., Wang, S., Zhou, J., 2013. Responses of the functional structure of soil microbial community to livestock grazing in the Tibetan alpine grassland. Glob. Chang. Biol. 19 (2), 637–648.

Yang, Y., Ashworth, A.J., DeBruyn, J.M., Willett, C., Durso, L.M., Cook, K., Moore, P.A., Owens, P.R., 2019b. Soil bacterial biodiversity is driven by long-term pasture management, poultry litter, and cattle manure inputs. PeerJ 7, e7839.

Yong-Zhong, S., Yu-Lin, L., Jian-Yuan, C., Wen-Zhi, Z., 2005. Influences of continuous grazing and livestock exclusion on soil properties in a degraded sandy grassland, Inner Mongolia, northern China. CATENA 59 (3), 267–278.

Zhang, F., Zheng, J., Bennett, J.A., Zhang, B., Wang, Z., Li, Z., Li, H., Wang, J., Han, G., Zhao, M., 2023. The legacy effects of grazing and precipitation variability on grassland productivity in a temperate steppe. Agric. For. Meteorol. 343, 109788.
Zhang, M., Wen, J., Wang, X., Zhao, C., Ma, Y., Ma, Y., Li, S., 2025. Rest-grazing in spring affects the soil microbial community mainly by altering plant diversity. J. Plant Ecol. 18 (1), rtae110.
Zhao, F., Ren, C., Shelton, S., Wang, Z., Pang, G., Chen, J., Wang, J., 2017. Grazing intensity influence soil microbial communities and their implications for soil respiration. Agric. Ecosyst. Environ. 249, 50–56.
Zhong, L., Du, R., Ding, K., Kang, X., Li, F.Y., Bowatte, S., Hoogendoorn, C.J., Wang, Y., Rui, Y., Jiang, L., Wang, S., 2014. Effects of grazing on N2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China. Soil Biol. Biochem. 69, 1–10.
Zhou, G., Zhou, X., He, Y., Shao, J., Hu, Z., Liu, R., Zhou, H., Hosseinibai, S., 2017. Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. Glob. Chang. Biol. 23 (3), 1167–1179.
Zhou, Z.C., Gan, Z.T., Shangguan, Z.P., Dong, Z.B., 2010. Effects of grazing on soil physical properties and soil erodibility in semiarid grassland of the Northern Loess Plateau (China). CATENA 82 (2), 87–91.