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Carbon and nitrogen in the topsoils of Inceptisols and Mollisols under native sage scrub and non-native grasslands in southern California

Tal Caspi [a], Leo Estrada [b], Anna V. Dowling [b], Erin Su [b], Maxim Leshchinskiy [b], André R.O. Cavalcanti [b], Edward J. Crane III [b], Colin R. Robins [a], Wallace M. Meyer III [b,*]

[a] W.M. Keck Science Department, Claremont McKenna, Pitzer, Scripps Colleges, 925 N. Mills Avenue, Claremont, CA 91711, USA
[b] Department of Biology, Pomona College, 175 W. 6th St., Claremont, CA 91711, USA

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ABSTRACT

Understanding how invasive plants influence terrestrial carbon (C) and nitrogen (N) budgets is important in the context of global climate change. In southern California, type-conversion, a process in which native California sage scrub is type-converted to non-native grassland, is thought to negatively impact total C and N storage in surface soil horizons. To better understand the extent to which type-conversion influences regional nutrient storage, we examined C and N concentration (%) and quantity (g/m^2), key soil properties, and microbial abundances and assemblages in sage scrub and non-native grassland habitats at three sites that represent varying environmental conditions. Type-conversion decreased soil C concentration, but did not influence C quantity. Differences between these two metrics were driven by a higher aggregate soil density in the non-native grassland habitat compared to the sage scrub habitat at one site. Contrary to previous studies, we found that type-conversion did not impact total N storage, even in a site previously found to have increased soil N quantities under sage scrub. Sage scrub habitats contained more active fungi, and differences in microbial assemblages were found between habitat types. Despite the vast number of microbial OTUs, habitats harbored unique communities of microbial taxa with some species consistently more abundant in one habitat type across sites. Our results demonstrate that type-conversion negatively impacts topsoil C concentrations, but accurate modeling of nutrient stocks requires consideration of the links between vegetation structure, soil properties such as soil density, and microbial communities that vary significantly across small spatial scales. Collectively, we demonstrate that invasive grasses alter microbial communities and reduce soil C storage capacity in the region.

1. Introduction

Determining how non-native species influence nutrient storage is crucial to our understanding of global carbon (C) and nitrogen (N) cycling (Bradley et al., 2006; Houghton, 2007; Ostle and Ward, 2012; Pinno and Wilson, 2011). In many systems, invasive grasses are dominant competitors that play important roles in nutrient storage dynamics by modifying abiotic and biotic factors and altering the natural disturbance regimes (D'Antonio and Vitousek, 1992; Mack and D'Antonio, 1998). Such alterations may have profound effects on ecosystem processes because changes in plant composition can also influence microbial communities, which are drivers of biogeochemical cycling (Jackson et al., 2002; Jobbágy and Jackson, 2000; Wardle, 2006; Wurst et al., 2012). While many studies have examined how grass invasion influences nutrient storage, conflicting outcomes indicate that impacts are dependent on ecological context (Pinno and Wilson, 2011). Thus, for many regions, our knowledge of how invasion by grasses affects terrestrial C and N budgets is limited.

Effects of invasion on nutrient storage in the California sage scrub ecosystem (hereafter sage scrub), a native low-elevation habitat in southern California dominated by drought deciduous shrubs, is still rudimentary (but see, Wheeler et al., 2016; Wolkovich et al., 2010). Sage scrub is undergoing largescale replacement by non-native grass species, a process known as type conversion (Cox et al., 2014; Talluto and Suding, 2008). Estimates indicate that sage scrub has declined by nearly 50% since the 1930s largely due to type-conversion (Riordan and Rundel, 2014; Rundel, 2007; Talluto and Suding, 2008). Reductions in fire return intervals are facilitated by grass invasion leading to a positive feedback with shorter fire return intervals as grass establishment enhances fire ignition probabilities(D'Antonio and Vitousek, 1992; Kimball et al., 2014; Talluto and Suding, 2008). Increased N deposition contributes by facilitating growth of non-native grasses and increasing their competitive ability (Kimball et al., 2014; Talluto and Suding, 2008). Together, these factors facilitate largescale type-conversion of sage scrub, resulting in long-lasting alterations in the region's

* Corresponding author.
E-mail address: wallace.meyer@pomona.edu (W.M. Meyer).

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vegetation community and structure (Mooney and Zavaleta, 2016; Rundel, 2007). Such changes can impact C and N cycling of the ecosystem in a number of pools (Ehrenfeld, 2003; Wheeler et al., 2016).

Soil is a major terrestrial pool of C, storing two to three times more C than aboveground biomass or litter components (Houghton, 2007; Wheeler et al., 2016). Total soil C is sensitive to both abiotic and biotic factors such as topography, soil texture, and plant species composition (Batjes, 2014; Jobbágy and Jackson, 2000; Pouyat et al., 2006; Wheeler et al., 2016). Less total N is stored in the soil than C, but soil N is equally important because fluxes between pools of N are largely controlled by soil microorganisms (Fowler et al., 2013; Ward, 2012). Surface soil horizons, which are typically generalized for sampling and discussion in the ecological literature as the top 10 cm of the A horizon or mineral soil profile, contain more microbial biomass than deeper soil horizons (Fierer et al., 2003; Lavahun et al., 1996) and are especially volatile with regard to C and N storage. Carbon and N in surface soil horizons are primarily controlled by soil mineralogy and organic matter inputs from plant growth, and balanced by respiration of the soil microbial community (Houghton, 2007). Bacterially-dominated soils promote rapid nutrient turnover and low nutrient storage (Manning, 2012; Wardle, 2002), whereas soils with higher abundances of fungi enhance soil C accumulation, because fungi use C more efficiently (Allison et al., 2005; Manning, 2012; Wardle, 2002). In addition, different habitats can harbor unique assemblages of microbial taxa with distinct functional characteristics (Fierer et al., 2012; Sigüenza et al., 2006). As such, it is essential to consider the complex links between vegetation structure, the microbial community, and soil C and N cycling when examining the effects of invasive grasses on sage scrub communities.

To better understand the impact of type-conversion on southern California surface soils, we examined differences in total C and total N storage in sage scrub and non-native grasslands at three representative sites across the region. While soils contain many key nutrients (P, S, Ca, Mg, Na, K, etc.), we use the term nutrient to refer exclusively to soil C and N. Sampling soil from both habitat types at each site, we (1) assessed the concentration and quantity of C and N stored in the uppermost mineral soil horizon (A horizon) and (2) tested whether microbial abundance and assemblages differed between habitat types. We also collected data on key soil properties in each habitat at each site to preliminarily examine how those properties may influence nutrient storage and assess differences in soil types. We hypothesized that sage scrub stores more total soil C and N than non-native grasslands. We also predicted that sage scrub soils would have increased fungal abundance and a higher fungi:bacteria ratio. By comparing total C, total N, and soil microbial assemblages between sage scrub and non-native grasslands at multiple sites spanning various environmental gradients, we demonstrate how type-conversion influences nutrient storage in the region and offer insight into the role of microbes in this key ecosystem process.

2. Methods

2.1. Study site

The sage scrub ecosystem is broadly characterized by a Mediterranean climate consisting of warm, dry summers and cooler, moist winters while also experiencing a gradient of increasing continentality moving from the coast inland (Mooney and Zavaleta, 2016). Within sage scrub, the climate gradient strongly influences the distribution of individual plant species (Riordan and Rundel, 2014; Rundel, 2007). Even though mean annual precipitation (MAP) and mean annual temperature (MAT) values can be nearly identical across the gradient, vegetation at the coast is buffered from climatic extremes, experiencing reduced temperature fluctuations and reduced drought stress compared to inland sites (Mooney and Zavaleta, 2016; Rundel, 2007). Work in the region by Bauer (1936) indicates that areas within 5 km from the coast experience double the humidity of more inland, higher-elevation sites. Along the coast to inland gradient, these climatic differences, topographic variation (primarily slope angle and aspect), and different soil types all contribute to and/or reflect distinct floristic assemblages responsible for the known "patchwork" or "mosaic" composition of the sage scrub community (Rundel, 2007). Most notably, evergreen shrubs, e.g., *Rhus integrifolia* and *Malosma laurina*, are more abundant at coastal sites, while drought deciduous shrubs like *Artemisia californica* and drought-tolerant species such as *Salvia apiana* become more frequent at inland sites (Rundel, 2007).

To better understand how type-conversion influences regional nutrient storage, we collected samples at three sites that span this coast to inland gradient in both Los Angeles and San Bernardino counties. The coastal site was in the Santa Monica Mountains (34.035948°, −118.804160°; MAP = 461 mm; MAT = 17 °C) while the most interior site was in the Crafton Hills Conservancy (34.037760°, −117.121647°; MAP = 324 mm; MAT = 18 °C), 155 km east (PRISM Climate Group, 2018). An intermediate site was the Robert J. Bernard Biological Field Station (hereafter Bernard Field Station; 34.109047°, −117.710374°; MAP = 514 mm; MAT = 18 °C), located 101 km east of the Santa Monica Mountains and 55 km west of Crafton Hills (see Fig. 1) (PRISM Climate Group, 2018). The Santa Monica Mountains site was only 2 km from the coast, whereas the Bernard Field Station and Crafton Hills sites were 55 km and 82 km from the coast, respectively, with the nearest coastline most often south of the study sites. Sites contained both sage scrub and type-converted grassland in close proximity (within 300 m) of one another to minimize the impacts of confounding variables such as variations in soil characteristics and microclimate within a site. Additionally, we chose sites in "protected areas" where differences in habitats were not associated with different contemporary land management practices such as grazing or disking for fire mitigation. Sage scrub was defined as areas dominated by native drought-deciduous and evergreen woody shrubs, most commonly *Artemisia californica*, and containing <10% cover by visual estimate of non-native species. Invasive grasslands were defined as containing <5% cover of native shrubs and were dominated instead by non-native European annual grasses *Bromus* spp. at inland sites. In addition to *Bromus* spp., the coastal site was co-dominated by *Brassica* spp., *Silybum marianum*, and *Centaurea melitensis*, highlighting the diversity of invasive annuals that can be abundant in disturbed areas in southern California.

Soils within these three sites exhibit a range of soil-landform characteristics representative of the sage scrub habitat diversity in southern California, and thus also differ in taxonomy. Soils at the coastal site are largely mapped by Soil Survey as Mollisols (Malibu or Boades series) formed in sandstone or shale parent materials (Soil Survey Staff, 2017). These soils are reported to have higher (e.g., 20–48%) clay content and CEC (~15 $cmol_c/kg$) than the inland sites where soils are much simpler, younger, and less developed Inceptisols and Entisols formed in alluvium (Bernard Field Station) and granitic residuum (Crafton Hills) with lower reported clay contents (8–20%) and CEC values (10–13 $cmol_c/kg$) (Soil Survey Staff, 2017). Soil Survey map units at Crafton Hills also include a clay-rich Alfisol (Ramona series) (Soil Survey Staff, 2017). Soil surveys are a useful first approximation, but their coarse 1:24,000 scale necessitates sampling at a finer-scale, especially in detailed studies of C and N. For instance, profile descriptions and sample analyses by Wheeler et al. (2016) found that the Bernard Field Station soils do not match and were more developed than any of the series suggested by existing survey units. Whatever the range in soil properties, comparisons between distinct soils are still warranted and even vital for regional study of type-conversion impacts on soil C and N storage under sage scrub, because sage scrub habitats span many distinct slope angles and parent materials. Moreover, Wheeler et al. (2016) found that soils were morphologically similar across the Bernard Field Station, and field observations for this study at the other

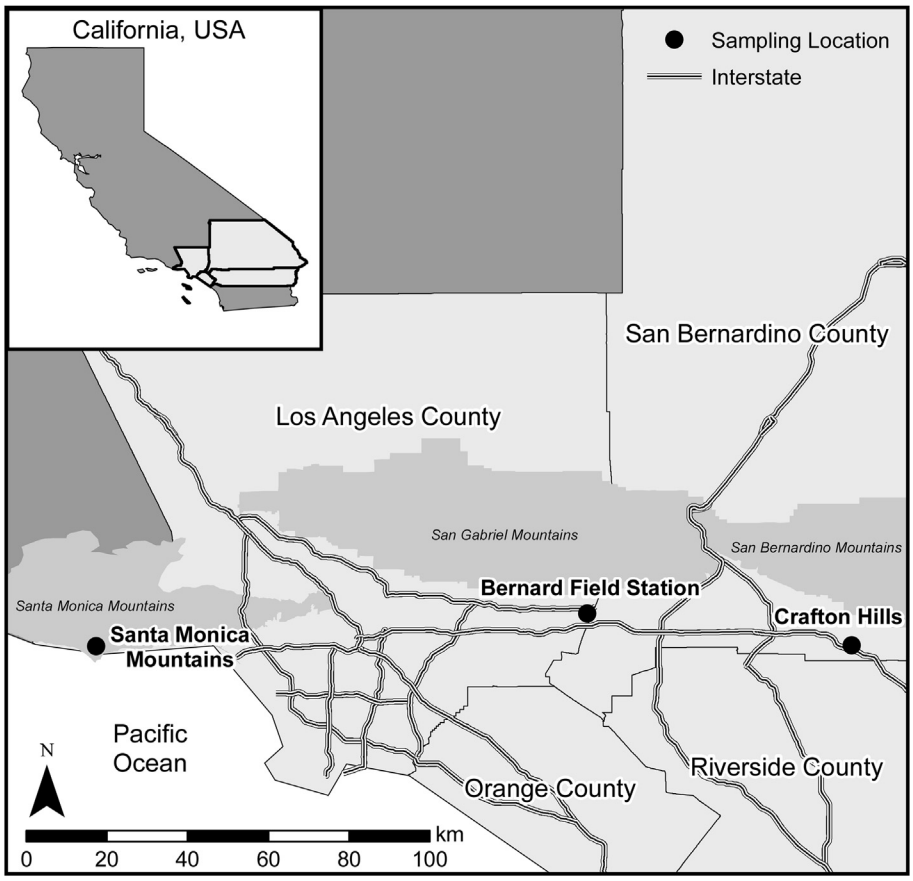

Fig. 1. Location of sites sampled from in southern California in the spring of 2016. Centroid coordinates of sites are: Santa Monica Mountains, 34.035984°, −118.804160°; Bernard Field Station, 34.109047°, −117.710374°; Crafton Hills, 34.037760°, −117.121647°.

two sites confirm that the A horizon does not greatly differ in appearance between habitat types.

2.2. Sample collection and processing

To compare total C, total N, soil properties, and microbial abundances, we collected soil from the top 10 cm of soil profiles at six sampling locations in both habitat types at each of the three sites in the spring of 2016 (March 25 through April 1). At each sampling location, we collected five different types of soil sample. First, we collected an intact soil aggregate from the very top of the mineral soil surface (i.e., the uppermost portion of the A horizon, immediately below the O horizon) to determine bulk density using a modified version of the clod method—a measure of soil density that excludes rock fragments > 2 mm diameter (Burt, 2004; Wheeler et al., 2016). Second, we gathered ~30 ml of soil from across the top ~10 cm of the soil profile (all within the A horizon) for analyses of percent C and N using an Elementar vario MICRO cube elemental analyzer (Elementar Mt. Laurel, New Jersey). We froze samples at −20 °C between sampling and analysis. Third, we collected ~250 ml of soil from the A horizon (again, sampling across the top ~10 cm) at each sampling location to send to Earthfort Laboratories (Corvallis, Oregon) to determine total and active bacteria and fungi. We sent samples immediately after collection. Total bacteria and fungi (μg/g) were determined through direct enumeration using microscopy. Bacteria were identified using the fluorescein isothiocyante method (Babiuk and Paul, 1970; Van Veen and Paul, 1979). Total fungal biomass was determined by converting width and length measurements. Active bacteria and fungi (μg/g) were quantified using direct microscopy after staining samples with fluorescein diacetate, which binds and fluoresces to metabolically active bacteria and fungi (Ingham, 1995; Schnurer and Rosswall, 1982). Fourth, we obtained 20 ml of soil from the A horizon at each sampling location for 16S and ITS amplicon metagenomics analysis, which involves the direct sequencing of the microbiomes in an environmental sample (Fierer et al., 2012). Finally, a composite sample was taken by mixing soil from the A horizons at all six sampling locations within each habitat at each site; these composite samples were sent to the UC Davis Analytical Laboratory to determine organic matter content, CEC, pH, and soil texture. For these last three sample types (soil for microbial abundance, metagenomics analysis, and soil properties), we also collected A horizon samples from a seventh sampling location in the non-native grassland in the Santa Monica Mountains (nutrient and microbial abundance data and metadata can be found on the KNB network: doi: 10.5063/F1000081).

2.3. Scaling and statistical analyses for nutrients and microbial abundances

Both the quantity (g/m^2) and the concentration (% per gram) of C and N in the uppermost mineral soil horizon (A horizon) were determined. Total C and N measurements (e.g., C and N quantities) were scaled-up to report grams of C and N in a meter squared. These values are reported as an area (m^2) but incorporate 10 cm of depth (soil A horizon) to better represent nutrient storage patterns across the landscape. This was calculated by multiplying C and N concentration by aggregate soil density of the investigated soil layer excluding rocks, as proposed for soil organic carbon by Poeplau et al. (2017) (also see Burt, 2004; and Wheeler et al., 2016). Each metric (quantity and concentration) provides a view into important aspects of nutrient storage dynamics. Concentration represents the balance between nutrient inputs and outputs from plant production and microbial activity in addition to soil properties important for storage such as CEC, organic matter content, and soil texture. Quantity incorporates soil nutrient concentration with aggregate soil density. With regard to the

consideration of total C versus organic C, none of the soils we investigated are mapped as containing secondary $CaCO_3$ nor carbonate-bearing parent materials, and all have acidic pH (Soil Survey Staff, 2017). None of the samples were found to react with hydrochloric acid. As such, we quantify total C in these soils assuming it is indicative of soil organic carbon alone.

To test for differences in total C and N concentrations and quantities and differences in total and active bacterial and fungal density between habitats, we ran multiple two-factor univariate PERMANOVA tests with site and habitat as factors. Each PERMANOVA test used a resemblance matrix constructed using Euclidean distances in the program PRIMER-E with the PERMANOVA+ add on (Anderson et al., 2008). Because we expected differences among sites, this design allowed us to test for differences between habitat types, while controlling for the variation expected among sites. One sample from sage scrub in the Bernard Field Station was considered an outlier because it had high concentrations of C and N and was removed, making comparisons between habitats more conservative. Following significant PERMANOVA results for the site x habitat interaction term, we conducted pairwise comparisons using one-factor PERMANOVA tests with habitat as the factor using data from each site ($\alpha = 0.017$ for multiple testing). To determine whether aggregate soil density differs between habitats within sites, we ran three one-factor PERMANOVAs using data from each site.

2.4. DNA extraction and sequencing

Approximately 0.25 g of soil from each sample was used for DNA extraction using MoBio PowerSoil DNA extraction kit following the manufacturer's instructions. Eluted DNA was stored at −20 °C before being sent for sequencing at Molecular Research LP (MR DNA; http://www.mrdnalab.com).

For each sample the following primers were used to amplify 16S bacterial DNA: 27Fmod (AGRGTTTGATCMTGGCTCAG) and 519Rmod (GTNTTACNGCGGCKGCTG); and fungal ITS sequences: ITS1F12 (GAACCWGCGGARGGATCA) and ITS2 (GCTGCGTTCTTCATCGATGC).

These primers (with a barcode on the forward primer) were used in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) - 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, with a final elongation step of 5 min at 72 °C. After PCR, the samples were pooled and a DNA library constructed using the Illumina TruSeq DNA library preparation protocol and sequenced using the Illumina MiSeq v3 2 × 300 bp sequencing platform following the manufacturer's guidelines (Illumina, San Diego, CA, USA). Paired-end reads were joined by MR DNA following q25 trimming of the ends, and sequences in the 3′-5′ orientation were reoriented into a uniform direction. The raw sequence reads are available at GenBank (accession number PRJNA398660).

2.5. OTU analyses

Sequences were assigned to 97% similar OTUs using QIIME (Caporaso et al., 2010). Sequences were quality filtered and barcodes removed, followed by removal of chimeric sequences using VSEARCH (Rognes et al., 2016) and assignment to OTUs. OTUs were assigned using the Greengenes database version 13.8 for the bacterial sequences, and the UNITE database version 7.1 (11/20/2016; https://unite.ut.ee/repository.php) for fungal sequences.

For the fungal sequences, following quality filtering but before chimera identification, we used the ITSx software (Bengtsson-Palme et al., 2013) to extract only the highly variable ITS1 region from the sequences. Only this region was used for OTU assignment, because inclusion of parts of the highly conserved, neighboring ribosomal genes can lead to misleading results (Bengtsson-Palme et al., 2013).

Following OTU assignment, we eliminated any taxonomically unassigned OTUs and the resulting BIOM table was imported into R (R Core Team, 2017) using the Phyloseq package (McMurdie and Holmes, 2013). All the following analyses were performed in R. Principal Coordinate Analysis was performed using the "ordinate" function of Phyloseq with Bray-Curtis and Binary (presence-absence) Bray-Curtis distances. Statistical significance of the differences in microbial assemblages between sites and habitat were determined using two-factor univariate PERMANOVA tests with site and habitat as factors using Bray-Curtis and Binary Bray-Curtis distances, implemented in the "adonis" function of the Vegan package (Oksanen et al., 2017). Statistical significances of the differences between habitats within each site were also calculated using one factor (habitat) PERMANOVAs. The "DESeq" function of the DESeq2 package (Love et al., 2014) was used to detect differentially abundant OTUs between habitats in each of the three sites. The phyloseq-formatted microbiome data was converted to a DESeqDataSet using the "phyloseq_to_deseq2" function of phyloseq. To initially explore if bacterial and fungal assemblages across sites respond in a similar way with regard to type-conversion, we listed the top 30 OTUs that statistically differ in abundance between the sage scrub and non-native grassland from each site and examined if identical OTUs made multiple lists.

3. Results

3.1. Soil properties

Soil properties differed among sites (Table 1) in a manner largely consistent with predictions based on climate, parent material, and topography. The exception was Crafton Hills, in which the gravelly sandy loam soils observed in this study did not match the Soil Survey map units that suggest a clay-rich Alfisol (Ramona series) at this site (Soil Survey Staff, 2017). Organic matter content and cation exchange capacity (CEC) were higher at the coastal site in both habitat types as compared to either inland site. The coastal site also had a finer soil texture than the inland sites, containing a higher proportion of clay relative to sand or silt. While few consistent trends were observed between habitats, variation in some soil properties between habitat types within certain sites does warrant comment. For example, CEC was approximately 1.5 times higher under the non-native grassland than sage scrub in the Santa Monica Mountains site, while the difference in CEC was much smaller at Crafton Hills. The opposite trend was observed at the Bernard Field Station: CEC was slightly higher under sage scrub than grassland. These discrepancies highlight the importance of assessing soil characteristics at smaller and more localized spatial scales than permitted through Soil Survey data alone and that assuming soil properties do not differ across small spatial scales within sites is unwarranted.

3.2. Soil nutrients

The topsoil in the sage scrub habitat contained a higher percentage of C than non-native grassland habitat (Pseudo-$F_1 = 5.54$, $P = 0.026$; Fig. 2). Averaged among all sampling locations from the three sites, A horizons under sage scrub contained 2.50% C, whereas A horizons under non-native grassland contained 1.95% C (over 20% less C than sage scrub). The two habitat types did not differ in total C (Pseudo-$F_1 = 1.14$, $P = 0.30$), as determined by the clod method, nor either measure of N (percent N: Pseudo-$F_1 = 0.83$, $P = 0.37$; total N: Pseudo-$F_1 = 0.10$, $P = 0.76$, Fig. 2). Site x habitat interactions were not significant (Fig. 2). Aggregate (clod) density was higher in the non-native grassland than sage scrub habitat in the Santa Monica Mountains (Pseudo-$F_1 = 8.54$, $P = 0.018$). Aggregate density did not differ between habitats at the Bernard Field Station (Pseudo-$F_1 = 0.04$, $P = 0.838$) nor Crafton Hills (Pseudo-$F_1 = 0.57$, $P = 0.459$).

3.3. Microbial abundance

Though sage scrub contained more active fungi than non-native grassland habitats (Fig. 3), most other microbiological metrics did not

Table 1
Soil properties from sage scrub and non-native grassland habitats at three sites in southern California.

Sample	Soil order[a]	pH	CEC	OM (%)	Soil texture		
					Sand (%)	Silt (%)	Clay (%)
Santa Monica Mountains CSS	Mollisol	5.17	26.4	7.53	37	33	30
Santa Monica Mountains NNG	Mollisol	5.66	41.6	6.95	25	28	47
Bernard Field Station CSS	Inceptisol	5.25	12.4	4.20	84	12	4
Bernard Field Station NNG	Inceptisol	5.68	8.2	1.94	79	16	5
Crafton Hills CSS	Inceptisol	5.12	14.9	4.47	65	26	9
Crafton Hills NNG	Inceptisol	5.65	19.1	4.74	63	25	12

[a] Soil Order determination is based on field observations, laboratory data, and comparisons to existing Soil Survey data (Soil Survey Staf, 2017), as described in the text.

differ between habitat types. For total fungi and active bacteria, however, there was a significant site x habitat interaction term (total fungi: Pseudo-F_1 = 3.95, P = 0.03; active bacteria: Pseudo-F_1 = 13.11, P = 0.0003), suggesting that the effect of type-conversion on these microbial abundances is contingent upon site (Fig. 3). Pair-wise comparisons examining differences between habitats within sites indicated that total fungi did not differ between habitats at any site after correcting for multiple testing (Santa Monica Mountains: Pseudo-F_1 = 2.36, P = 0.140; Bernard Field Station: Pseudo-F_1 = 2.44, P = 0.145; Crafton Hills: Pseudo-F_1 = 6.17, P = 0.049). Active bacterial concentrations were higher in the sage scrub habitat in the Santa Monica Mountains (Pseudo-F_1 = 29.54, P = 0.004), but did not differ between habitats at the Bernard Field Station (Pseudo-F_1 = 2.49, P = 0.135) or Crafton Hills (Pseudo-F_1 = 0.73, P = 0.417). Total bacteria did not differ between habitat types (Fig. 3). While active bacterial concentrations were higher in the sage scrub habitat in the Santa Monica Mountains, that increase did not influence active fungi:bacteria ratios. For active fungi:bacteria ratios, habitat was not significant (Pseudo-F_1 = 0.77, P = 0.57) but the site x habitat interaction was (Pseudo-F_2 = 4.62, P = 0.02). Pair-wise comparisons revealed that sage scrub habitat had a higher active fungi:bacteria ratio than non-native grassland at the Santa Monica Mountains site (Pseudo-F_1 = 8.52, P = 0.019; Fig. 3), since differences in fungal abundances between the two habitats were greater than differences in bacterial abundance (Fig. 3). Active fungi:bacteria ratios did not differ between habitats at the Bernard Field Station (Pseudo-F_1 = 2.31, P = 0.171) or Crafton Hills (Pseudo-F_1 = 0.26, P = 0.601). Total fungi:bacteria ratios did not differ among habitats (Pseudo-F_1 = 2.03, P = 0.16).

3.4. Microbial assemblages

In total, we recorded 46,913 bacterial OTUs and 2952 fungal OTUs. Bacteria OTU richness ranged from 20,595 to 29,393 OTUs among sites and from 5095 to 9256 OTUs among samples. Fungal OTU richness ranged from 1641 to 1935 OTUs among sites and 383 to 802 OTUs among samples.

As expected, bacterial and fungal assemblages differed among sites using both Bray-Curtis and Binary (presence-absence) Bray-Curtis distances to compare groups (bacteria Bray-Curtis: F_2 = 4.62, P = 0.0001; fungi Bray-Curtis: F_2 = 4.34, P = 0.0001; bacteria Binary Bray-Curtis: F_2 = 3.82, P = 0.0001; fungi Binary Bray-Curtis: F_2 = 3.13, P = 0.0001; Fig. 4).

Using both measures (Bray-Curtis and Binary Bray-Curtis), bacterial and fungal assemblages differed between habitat types (bacteria Bray-Curtis: F_2 = 4.26, P = 0.0001; fungi Bray-Curtis: F_2 = 4.53, P = 0.0001; bacteria Binary Bray-Curtis: F_2 = 3.54, P = 0.0001; fungi Binary Bray-Curtis: F_2 = 2.95, P = 0.0001), suggesting that habitats differed with regard to both the composition of the bacterial species and their

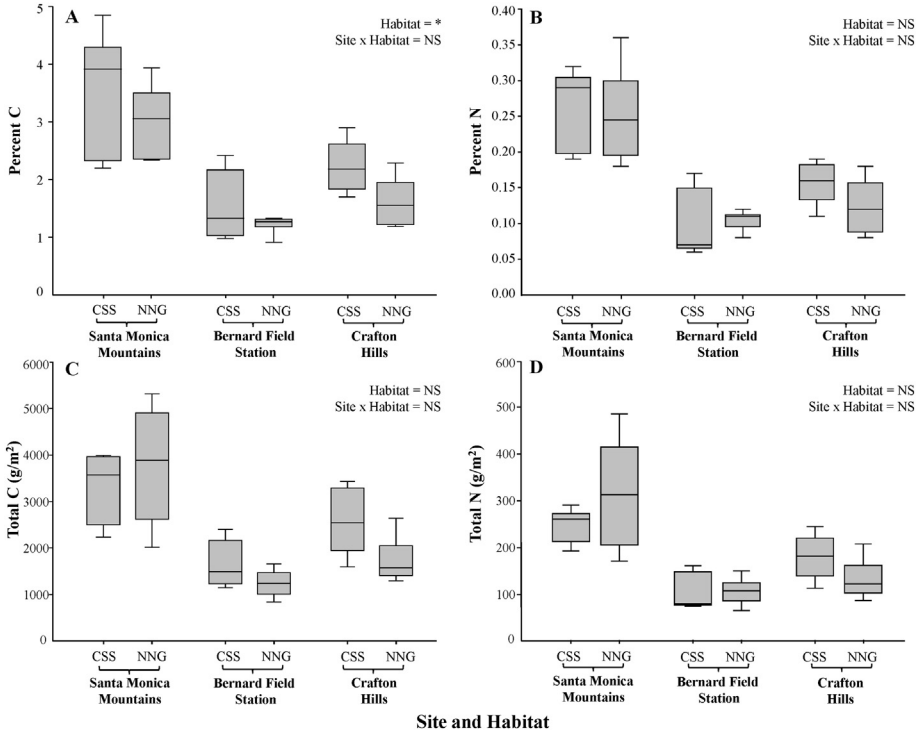

Fig. 2. Concentration (%) C (A) and N (B), and quantity (g/m^2) of C (C) and N (D) under sage scrub and non-native grassland habitats at three different sites in southern California. * = P-value < 0.05; NS = P-value > 0.05.

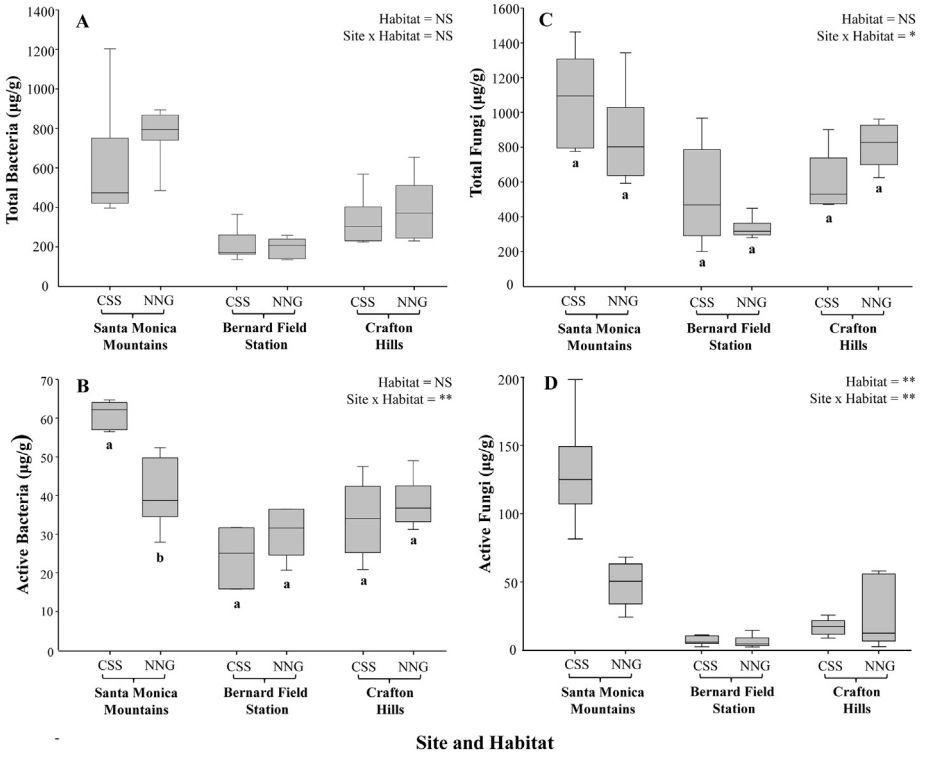

Fig. 3. Total bacteria (A), total fungi (B), active bacteria (C), and active fungi (D) under sage scrub and non-native grassland habitats at three different sites in southern California. * = P-value < 0.05; ** = P-value < 0.01; NS = P-value > 0.05. Pairwise comparisons were run following significant site x habitat interaction. Different letters denote significant differences in microbial concentrations between habitats within a site ($\alpha < 0.017$).

relative abundance. Within each site, sage scrub and non-native grassland had distinct microbial assemblages (Table 2). Among sites, there was overlap between the top thirty species/OTUs most different between sage scrub and non-native grassland in terms of abundance (Appendix A). For fungi, one unidentified OTU was more abundant in the non-native grassland at all three sites. Three other fungi OTUs, *Chytridiomycota* sp. 1, *Eurotiomycetes* sp. 1, and *Cladophialophora* sp. 5, were on the top 30 list at two sites, with *Chytridiomycota* sp. 1 more abundant in non-native grassland and *Eurotiomycetes* sp. 1 and *Cladophialophora* sp. 5 more abundant in sage scrub. For bacteria, only

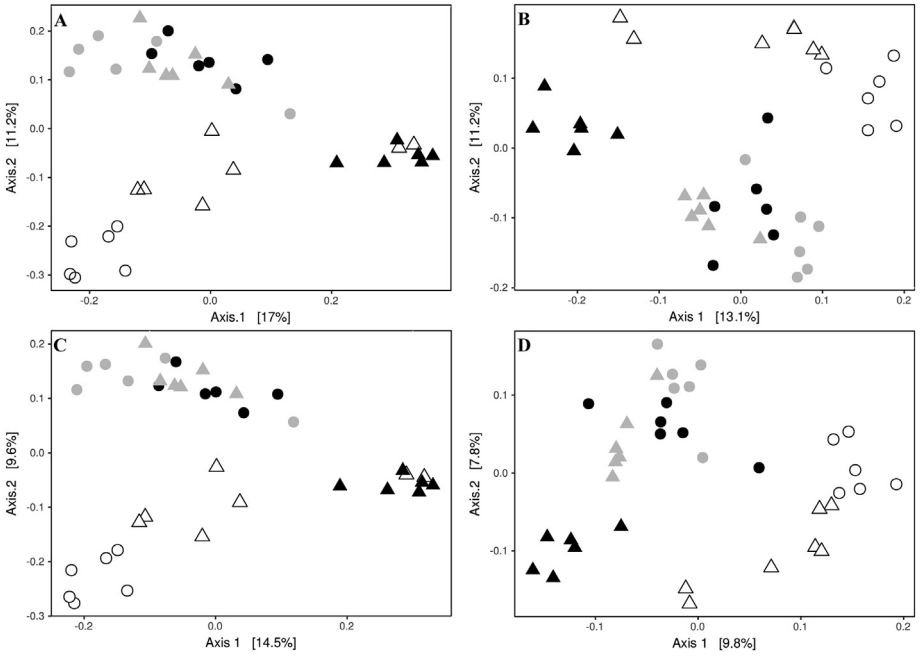

Fig. 4. PCoA ordination of sampling locations according to the relative abundance and composition of the microbial community in the surface soil horizon in each sample. Bacterial and fungal assemblages based on relative abundance are depicted in A and B, respectively. Bacterial and fungal assemblages based on presence-absence (composition) are depicted in C and D, respectively. Similarity determined using the Bray Curtis similarity coefficient. Circles represent sage scrub habitat and triangles represent non-native grassland. The Santa Monica Mountains are white, the Bernard Field Station is gray, and Crafton Hills is black.

Table 2
Results for pairwise tests comparing differences in microbial assemblages between adjacent sage scrub and non-native grasslands at three sites in southern California. Similarity matrices were created using the Bray Curtis coefficient. For each taxa, two separate analyses were run: one analysis used relative-abundance of OTU sequences, and another used OTU presence-absence data. Significant differences are asterisked.

		Santa Monica mountains				Bernard field station				Crafton hills			
		Relative abundance		Presence-absence		Relative abundance		Presence-absence		Relative abundance		Presence-absence	
Source	df	Pseudo-F	P	Pseudo-F	P	Pseudo-F	P	Pseudo-F	P	Pseudo-F	P	Pseudo-F	P
Bacteria	1	4.1847	0.0004*	3.4127	0.0005*	2.2117	0.0037*	1.9667	0.0043*	4.3279	0.0023*	3.4358	0.0021*
Fungi	1	3.3242	0.0004*	2.5396	0.0009*	2.5535	0.0033*	1.9352	0.0036*	3.6652	0.0016*	2.4449	0.0030*

one OTU, *Skermanella* sp. 3 was on more than one list of the 30 statistically significant OTUs, with higher abundance in non-native grassland than sage scrub at both the Santa Monica Mountains and Bernard Field Station. To provide perspective, the probability that a single fungal OTU would end up on the top 30 list of two or three sites is ≤0.028% and 0.0004%, respectively. Probabilities for bacteria OTUs to appear on multiple lists are effectively zero due to the high number of OTUs.

4. Discussion

4.1. Effects of type conversion

Our study underscores the importance of understanding how changes in both plant composition and differences in soil density influence nutrient storage. While C concentrations were over 20% higher in sage scrub than non-native grasslands, C quantity did not differ between habitats. Differences between these two metrics were driven by a higher aggregate density in the non-native grassland habitat compared to the sage scrub habitat in the Santa Monica Mountains site. Soil aggregate density did not differ between habitats at the inland sites (Bernard Field Station and Crafton Hills), and patterns of C storage were consistent with the results of Wheeler et al. (2016), who studied nutrient quantities at the Bernard Field Station three years earlier. Thus, type conversion negatively affects total soil C concentrations, reducing bulk nutrient storage capacities throughout the region. However, accurately modeling C quantities requires understanding local variability in soil density, which can be significant over small distances. As such, we argue that reporting both metrics is important because differences in soil density may obscure the impacts of type conversion if researchers only report total C.

In contrast to Wheeler et al. (2016), who found that sage scrub stored more N than non-native grassland, type conversion did not influence N concentration or quantity in this study. *Bromus* spp. invasions have been associated with increases (Hooker et al., 2008; Norton et al., 2004), decreases (Sperry et al., 2006), and no changes (Rau et al., 2011) in soil N in a variety of systems. In addition to site-specific differences, N concentrations may also be changing temporally. For example, Wheeler et al. (2016) found increased N storage in sage scrub at the Bernard Field Station in the spring of 2013, but we observed an opposite trend in our study at the same site in the spring of 2016. In the years between these findings southern California experienced an exceptional drought (Mann and Gleick, 2015), which may have impacted N storage dynamics. As such, further studies should examine the extent to which type conversion and interannual variation, particularly interannual differences in water availability, influence N storage in the region.

Nutrient concentrations in surface soil horizons are strongly influenced by soil microbial respiration (Houghton, 2007). Greater fungal domination has been shown to increase soil C storage (Kandeler et al., 2008) as fungi metabolize substrates more efficiently than bacteria (Allison et al., 2005), having lower respiration rates per unit biomass (Lipson et al., 2005). Because soils with higher abundances of fungi relative to bacteria enhance soil C accumulation (Allison et al., 2005; Manning, 2012; Wardle, 2002), we hypothesized that sage scrub habitats have higher concentrations of fungi and higher fungi:bacteria ratios. Consistent with our hypothesis, active fungal concentrations were higher in sage scrub habitat, which had the greatest percent C. Additionally, fungi:bacteria ratios were higher in the sage scrub habitat in the Santa Monica Mountain site, which stored the most nutrients. At inland sites, active fungi:bacteria ratios did not differ between sage scrub and non-native grassland habitats suggesting that differences in C concentrations at these sites are not associated with differences in the ratios of currently active fungi relative to bacteria. Total fungi:bacteria ratios also did not differ between habitats, indicating that nutrient storage, particularly C storage, between habitats may not be well characterized by these ratios.

In addition to fungal and bacterial abundances, unique assemblages of microbial taxa may differentially influence nutrient storage dynamics (Fierer et al., 2012; Sigüenza et al., 2006). In contrast to Karst et al. (2013), but consistent with Sigüenza et al. (2006), our study found that sage scrub and non-native grasslands harbor distinct microbial assemblages. We were surprised to find clear evidence that type-conversion produced similar changes in microbial composition between habitats across the three sites. By examining patterns of only the top 30 OTUs with the most statistically significant differences between habitat types at each site, we found one fungal OTU shared by all three sites and three fungal OTUs present at two sites. Given the magnitude of OTU diversity within sites and habitats, any overlap suggests that type-conversion may cause directional change in fungal communities, with sage scrub and grassland habitats each supporting different assemblages. Bacterial assemblages were also distinct between habitats, but had fewer of the top 30 statistically significant OTUs in common among sites as compared to fungi. This is expected as bacterial OTU diversity was approximately 16 times higher than fungal OTU diversity, reducing the already improbable likelihood that identical OTUs would make it onto multiple lists. Though the taxonomic structure of soil microbiomes in different habitat types cannot predict functional attributes related to nutrient storage, metagenomic sequencing provides a pathway for gaining insight into the roles of microbial communities in individual soil types (Fierer et al., 2012). Soil microbial communities are often altered by invasive plant species (Ehrenfeld, 2003; Hawkes et al., 2005; Sigüenza et al., 2006) and changes in microbial communities directly affect nutrient cycling rates (Hawkes et al., 2005). While further research is required to determine whether differences in microbial assemblages between native and non-native habitats in southern California influence nutrient storage, our results support the idea that each habitat type hosts unique microbiomes. This study provides (1) a list of microbial taxa that research might initially focus efforts on to explore differences in ecosystem function, and (2) confirmation that soil microbiomes differ between habitat types, a foundational first step for manipulative studies to examine functional differences between soils in the two habitats.

Though invasion in southern California is typically dominated by non-native grass species, other invasive annuals have become increasingly common in disturbed areas (Bell and Muller, 1973; Keeley, 2014; Lambert et al., 2010), and may impact ecosystem processes with different patterns. *Brassica* spp. in particular, which co-dominates the invasive habitat at the Santa Monica Mountains site, is known to alter microbial communities by inhibiting mycorrhizal fungal growth and spore germination (Schreiner and Koide, 1993), possibly further impacting the fungal colonization of other neighboring plants (Lankau

and Strauss, 2008). Whether or not differences in habitats observed at this site and/or differences between this site and the inland sites are related to the allelopathic effects of *Brassica* spp. is unknown, but further studies should consider whether and how the impacts of regional type-conversion change with the composition of the invading plant community.

4.2. Differences among sites

Overall, nutrient storage differed among sites, with the Santa Monica Mountains storing the most C and N and the Bernard Field Station storing the least. These differences demonstrate that nutrient storage capacity varies significantly among sites across the region. While our study was conducted at only three sites, preliminary findings suggest that clay content, CEC, and organic matter content all varied in accordance with predictions based on soil forming factors and regional soil taxonomy. With clays and organic matter typically providing the bulk of a soil's CEC, these metrics naturally co-correlate with total C and total N. A higher soil organic matter content, clay content, and thus, CEC, in the Santa Monica Mountains reflects greater moisture, greater soil profile development and a greater preponderance of Mollisols than the more inland, Inceptisol-dominated sites. Site-specific factors related to environmental conditions throughout the region vary, distinctly influencing soil profile development, plant growth, and, thus, nutrient storage capacity at each site. Ultimately, while our results preliminarily suggest that coastal sites may store more C and N than inland sites, our study was limited to a small sample size from surface horizons only, and greater replication is required to develop powerful, predictive modeling of C and N concentrations across the region.

4.3. Conclusions

Our results demonstrate that type conversion negatively impacts soil C concentrations, reducing C concentrations by approximately 20%. Our estimations of the effects of invasion are conservative because our study exclusively sampled soil surface horizons (A horizons). Shrubs have deeper root profiles than grasses and vertical soil C distributions may extend much deeper under shrublands (Jobbágy and Jackson, 2000). In addition, more C and N are stored in other components of the sage scrub habitat including aboveground vegetation and litter (O horizons) (Wheeler et al., 2016). In contrast to C, N storage was not found to be influenced by type-conversion. However, future studies should investigate the extent to which N budgets vary temporally and what factors influence temporal changes in each habitat type. Consistent with our hypothesis, sage scrub had higher fungal concentrations than non-native grasslands. Active fungi:bacteria ratios were higher in sage scrub at only one site, and total fungi:bacteria did not differ between habitats, indicating that these ratios may not be good indicators of nutrient storage in these habitats. Microbial assemblages differed between habitat types, indicating that grass invasion significantly alters soil microbial communities. Though the functional implications of these shifts are currently unknown, characterizing the soil microbiomes in the two habitats provides foundational information for future studies to elucidate how differences influence nutrient storage and other ecosystem processes. While more detailed quantification of links between vegetation structure, soil properties, and microbial communities is essential to more precisely capture the effects of invasive species on regional nutrient storage dynamics, our results highlight that invasion of *Bromus* spp. grasses negatively influence C storage throughout the region.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.geodrs.2018.e00172. These data include the Google map of the most important areas described in this article, and a table highlighting bacterial and fungal OTUs with the most significantly different abundace between sage scrub and non-native grassland habitats.

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