Soil Biological and Chemical Properties in Restored Perennial Grassland in California

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Abstract

Restoration of California native perennial grassland is often initiated with cultivation to reduce the density and cover of non-native annual grasses before seeding with native perennials. Tillage is known to adversely impact agriculturally cultivated land; thus changes in soil biological functions, as indicated by carbon (C) turnover and C retention, may also be negatively affected by these restoration techniques. We investigated a restored perennial grassland in the fourth year after planting *Nassella pulchra, Elymus glaucus,* and *Hordeum brachyantherum* ssp. *californicum* for total soil C and nitrogen (N), microbial biomass C, microbial respiration, CO_2 concentrations in the soil atmosphere, surface efflux of CO_2, and root distribution (0- to 15-, 15- to 30-, 30- to 60-, and 60- to 80-cm depths). A comparison was made between untreated annual grassland and plots without plant cover still maintained by tillage and herbicide. In the uppermost layer (0- to 15-cm depth), total C, microbial biomass C, and respiration were lower in the tilled, bare soil than in the grassland soils, as was CO_2 efflux from the soil surface. Root length near perennial bunchgrasses was lower at the surface and greater at lower depths than in the annual grass–dominated areas; a similar but less pronounced trend was observed for root biomass. Few differences in soil biological or chemical properties occurred below 15-cm depth, except that at lower depths, the CO_2 concentration in the soil atmosphere was lower in the plots without vegetation, possibly from reduced production of CO_2 due to the lack of root respiration. Similar microbiological properties in soil layers below 15-cm depth suggest that deeper microbiota rely on more recalcitrant C sources and are less affected by plant removal than in the surface layer, even after 6 years. Without primary production, restoration procedures with extended periods of tillage and herbicide applications led to net losses of C during the plant-free periods. However, at 4 years after planting native grasses, soil microbial biomass and activity were nearly the same as the former conditions represented by annual grassland, suggesting high resilience to the temporary disturbance caused by tillage.

Key words: carbon retention, CO_2 emissions, grassland restoration, *Nassella pulchra*, soil fertility, soil microbial biomass.

Introduction

Soil organic matter, nutrients, and biological activity contribute to ecosystem-level processes and are important for productivity, community structure, and fertility in terrestrial ecosystems. In many restoration projects, the main focus has been on the establishment of native plant species and creation of plant communities that closely resemble those of undisturbed native vegetation (Zedler 2001; Walker & del Moral 2003). Ecological impacts of restoration procedures on soils can directly affect plant community composition or system-level functions such as nutrient cycling and carbon (C) retention (Packard & Mutel 1996; Cione et al. 2002; De Deyn et al. 2003). Monitoring changes not only in vegetation but also in soils portrays the overall success of the restoration process more accurately.

Native perennial bunchgrasses dominated California grasslands prior to European settlement, but non-native annual grasses were able to outcompete the native perennial grasses after overgrazing and drought (Burcham 1957; Jackson 1985). Lowland areas in California now largely support annual grassland, which is mostly composed of non-native annual grasses from the Mediterranean Basin interspersed with some co-occurring native herbaceous species (Huenneke 1989). The existing relict sites of California grassland with perennial bunchgrasses are limited in extent, and the reasons for their resistance to invasion by the non-native annual grasses are not clearly understood. Although these sites provide a meaningful example of the target conditions sought in native grassland restoration projects, little is known about the soil biology throughout the profile in relict native grasslands. In the surface layer, however, relict native perennial grasslands had different soil microbial community composition but higher or equal amounts of soil microbial biomass C and equal amounts of total soil C and nitrogen (N) compared to annual grasslands (Steenwerth et al. 2003).

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Restoring native perennial bunchgrasses in California's non-native annual grassland has been notoriously difficult (White 1967; Heady 1977). A successful method has been to use agricultural techniques such as tillage and herbicide for 2–3 years to control exotic annuals before seeding with native perennials (Stromberg & Kephart 1996). Such measures have a strong impact on soil biology and biochemistry (Doran 1980; Follett & Schimel 1989; Aon et al. 2001). Plowing formerly undisturbed soil reduces microbial biomass levels in the upper soil layers and destroys the natural stratification of biological activity in the tilled layer (Woods 1989; Aslam et al. 1999). The stability of soil aggregates declines rapidly after tillage, altering soil structure and organic matter retention (Lynch and Bragg 1985; Francis et al. 2001). Moreover, cultivated soils in the Central Coast region of California tend to have lower total and labile C than grasslands (Steenwerth et al. 2003), due to both lower organic matter inputs and increased soil C and N mineralization rates due to tillage (Calderón & Jackson 2002).

Herbicides used in the restoration process can result in a broad spectrum of effects on soil biology depending on the active agent. Glyphosate, the active agent of Roundup, is reported to stimulate microbial activity without affecting the size of microbial biomass and appears to provide a labile C source for soil microbes, ensuring rapid degradation of the herbicide in soil (Haney et al. 2000).

As mandated for agricultural sustainability, restoration goals should enhance nutrient cycling and soil C storage as well as the conservation of the soil biological processes present in the undisturbed ecosystem (Beese et al. 1994; Doran & Linn 1994; Potthoff & Beese 2000). This will increase the potential for nutrient retention and prevention of C losses. In this context the size of the soil microbial biomass is a more sensitive indicator for biological changes, C retention, and soil fertility than total soil organic matter, which is largely composed of nonlabile compounds (Beck 1984; Jenkinson 1988, 1990).

In this study, the objectives were to determine (1) if soil properties and soil biology change during the process of restoring native vegetation and if so, (2) are soil functions restored in a manner conducive to retaining soil C and biological activity by soil microorganisms at various depths in the soil profile?

Materials and Methods

Site and Treatments

Our investigations took place in April 2002, during the season of peak biological activity, at the UC Hastings Natural History Reservation in the foothills of the Santa Lucia Mountains in Upper Carmel Valley (long 121°0′31″W, lat 36°30′12″N). The study site was on a Sheridan coarse sandy loam soil (Coarse-loamy, mixed, thermic Pachic Haploxerolls; Cook 1978) located on a level area that had been farmed between 1865 and 1937. From 1937 to 1996 the site supported annual grassland. The climate of this region is Mediterranean, with small annual amplitudes in daily mean temperatures (15.5°C in summer and 13.0°C in winter; Cook 1978). Mean annual precipitation ranges between approximately 350 and 500 mm and occurs from September to May.

In November 1995, the restoration process started by tilling a 50 × 20–m^2 plot within the annual grassland (Fig. 1), which has been monitored for plant species composition since 1971 (Stromberg & Griffin 1996). First, soil was intensively disked to 45-cm depth. Later, each time annual seedlings colonized the field, it was rototilled to remove the annual plants before they could set seed. The harrow was adjusted to a depth of 25 cm. Tillage thus occurred four times per year during the wet season. In addition, glyphosate (Roundup Grass/Weed Killer, Ortho, Columbus, OH, U.S.A.) was applied once a year. The herbicide was usually sprayed in April to remove all sprouting vegetation when soil moisture did not allow vehicles to enter the plot. These procedures were effective in eliminating the annual seed bank in soil. By the second year, the cover of annuals between tillage events was less than 10%.

In December 1997, native perennial bunchgrasses were sown in a 600-m^2 area after the plot was harrowed (Fig. 1). *Nassella pulchra* (Agrostideae tribe), *Elymus glaucus* (Hordeae tribe), and *Hordeum brachyantherum* ssp. *californicum* (Hordeae tribe) were seeded at 50, 75, and 38 kg/ha, respectively. At the two far ends of the plot, smaller areas (200-m^2) were kept free of plants by rototilling and Roundup applications two or three times each year. No irrigation or fertilizer was subsequently applied in any treatment plots. Spatially, the restored perennial stand consisted of two types of microenvironments: zones close to the planted bunchgrasses and zones between the plants.

Sampling in April 2002 consisted of the following:

(1) tilled annual grassland that was plant free for 6 years using tillage and Roundup herbicide;
(2) old field annual grassland, left fallow 65 years ago;
(3) 4-year-old restored perennial grassland that was created after tilling annual grassland and planting with native bunchgrasses:
   (a) Sampling zone between bunches of *N. pulchra*, at least 20 cm from a bunchgrass.
   (b) Sampling zone very close to *N. pulchra* plants at the perimeter of the bunchgrass crown.

Sampling and Analysis

Investigations were carried out as an intensive 3-day sampling campaign, including the monitoring of the current vegetation (aboveground biomass by species as well as total root biomass and length), soil physical properties (bulk density), soil chemical properties (total C and N content, CO_2 concentrations in the soil atmosphere, and CO_2 efflux from the soil surface), and soil biological properties (soil microbial biomass C and soil respiration).

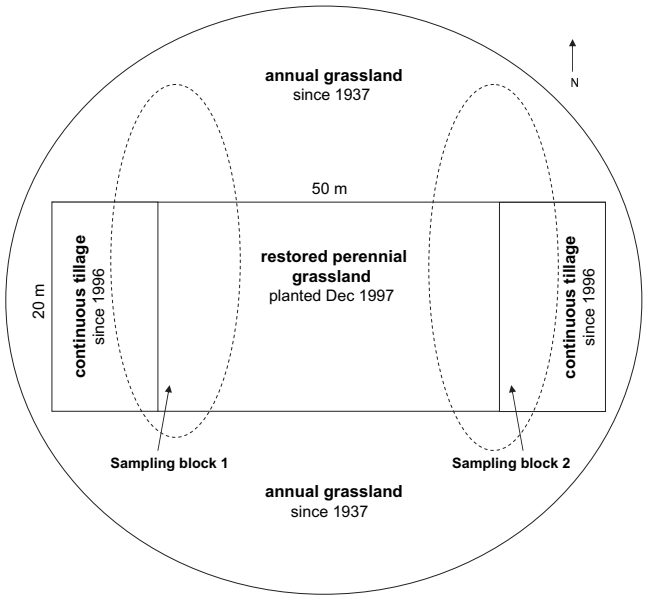

Figure 1. Schematic diagram of the field plots at Hastings Reserve in Carmel Valley, California, showing the three different management treatments and the two areas (blocks) of soil sampling.

Sampling was done in two blocks (Fig. 1). Root and soil measurements were taken in four soil layers (0–15, 15–30, 30–60, and 60–80 cm). Every treatment and depth combination was sampled with four randomly chosen replicates (two in each block).

Aboveground vegetation was clipped in 20×20–cm^2 frames above the soil blocks that would be subsequently sampled for soil parameters. Biomass of species was determined after drying for 48 hr at 40°C. Plant species identification was according to Hickman (1993).

From the sides of two soil pits per treatment per sampling block, soil blocks ($15 \times 15 \times 15$ cm in size) were removed from the midpoint of each sampling depth. The soil was gently mixed and subsampled for roots and soil properties.

Thin steel tubes with an inner diameter of 2 mm and a solid pointed tip were pushed into the soil. They had perforations along 4 cm of their length at 68–72, 43–47, or 20.5–24.5 cm. Tubes were sealed with rubber septa to sample soil gas at different depths. The perforation of the steel tubes was protected from soil contamination with a dense steel screen. The upper soil layer was sampled using cannulated needles (7.5-cm depth). Gas samples were taken using commercial syringes (5 ml) and vacutainers (3 ml) (Becton Dickinson, Franklin Lakes, NJ, U.S.A.). Gas samples were analyzed for CO_2 concentrations using a CO_2 analyzer (Horiba PIR-200, Riverside, CA, U.S.A.) with infrared detection.

CO_2 surface efflux was determined using the closed chamber method modified from Rolston (1986). The gas sampling caps consisted of a central steel cylinder with a complete coating of Styrofoam on all sides. The free bottom rim was pushed about 2 cm deep in the soil. A steel tube, reaching the volumetric center of the air space, was sealed with a rubber septum on the top of the cap. The cylinders were 12 cm in diameter and 11 cm in height. Efflux was calculated from the CO_2 enrichment after 15 minutes compared to an ambient reference taken at the site 10 cm above the ground. Sampling and analyses were carried out as described for the soil gas CO_2 concentrations.

Roots were immediately washed from a 200-g subsample of soil, and live roots were separated from senescent and decaying roots and organic matter fragments by floating and with forceps. Root length was recorded using a Comair root scanner (Commonwealth Aircraft Corporation, Ltd., Melbourne, Australia). Root biomass was recorded as root dry weight after drying at 40°C for 48 hr.

Total soil C and N were determined by the combustion gas analyzer method (Pella 1990). Soil microbial biomass C was measured by fumigation extraction (Brookes et al. 1985; Vance et al. 1987). Field-moist soil of 50-g dry weight was split into two portions (25 g for the fumigated and 25 g for the nonfumigated treatment), extracted by oscillating shaking at 250 revolutions/minute with 100 ml 0.5 M K_2SO_4, and filtered through a washed folded paper filter (Whatman No. 3, Springfield Mill, Maidstone, Kent, U.K.). Organic C in the K_2SO_4 extracts was measured using diluted extracts (1:10) and a Phoenix 8000 automatic analyzer (Dohrmann [Tekmar-Dohrmann], Manson, OH, U.S.A.) according to the method of Wu et al. (1990). Soil microbial biomass C was calculated from the relationship: biomass $C = E_C / k_{E_C}$ (E_C = [organic C extracted from fumigated soil] − [organic C extracted from nonfumigated soil]; $k_{E_C} = 0.45$) (Wu et al. 1990; Joergensen 1996).

Soil respiration was determined for sieved (4 mm) soil samples from which all visible plant (root) materials were removed. Forty-eight hours after sieving, 200-g field-moist soil samples were placed into 250-ml jars, which were gas tight and had rubber septa for gas sampling in the lid. Gas samples were taken after an incubation time of 1 hr at 25°C under darkness. Sampling and analyses followed the same procedure as described for the gas sampling in the field. Lab air was taken as the ambient control.

To obtain bulk density, samples were taken at the midpoint of each layer using 6-cm-deep brass rings with a volume of 332 cm^3 (4.5–10.5 cm representing the 0- to 15-cm layer, 19.5–25.5 cm representing the 15- to 30-cm layer, 42–48 cm representing the 30- to 60-cm layer, and 67–73 cm representing the 60- to 80-cm layer). Bulk density was calculated on an oven dry basis (105°C for 48 hr).

Soil data were analyzed by three-way analysis of variance (ANOVA) for the factors treatment, sample block, and depth. Results are presented as average values of the four replicates with standard deviation or as indicated. For comparison of means the multivariate Tukey HSD (Honest Significant Difference) test was applied to the soil and the plant biomass data (Statistica, Statsoft, Tulsa, OK, U.S.A.). The level for significant differences was 0.05. No field replications of this setup or of any of the treatments were available for destructive sampling, and thus this study was limited to one field site representing typical restoration procedures in coastal annual grassland. Hence, statistics were applied to sample replicates and not on field replicates.

Results

Plant Species and Plant Biomass

The restored perennial grassland near and between bunchgrasses and the adjacent annual grassland supported different amounts of both total plant biomass and relative biomass of native plant species even though total species richness was similar (Table 1). Total aboveground plant biomass was significantly higher near the bunchgrasses than between the bunches and in annual grassland. It tended to be lower between the bunches of perennials than in the annual grassland. Of the total biomass, native plant species composed 82% of the species in the near bunchgrass areas, 32% between the bunchgrasses, and 14% in the annual grassland. *Nassella pulchra* was the main perennial grass in the sampled zones, with individuals of *Elymus glaucus* intermixed in some plots. These species represented 89% of native plant biomass in the "near bunchgrass" area, whereas *Lupinus nanus* and *Lupinus bicolor* (Fabaceae) represented 97% of native plant biomass in the "between bunchgrass" zone.

The same number of non-native grass species occurred at all sites, but the annual grassland contained more non-native grass biomass than "between" and "near" bunchgrasses in the restored perennial grassland, which mainly was due to significantly higher biomass of *Bromus hordeaceus* (Festuceae tribe) in the annual grassland (Table 1). The restored perennial grassland had similar numbers of native forbs as the annual grassland. Biomass of non-native forbs represented 45% of all annual grassland forbs. This was much higher than the proportion of non-native forbs in the two restored perennial grassland zones. The significantly larger biomass of *Erodium cicutarium* (Geraniaceae) and *Cerastium glomeratum* (Caryophyllaceae) contributed to this high proportion of non-native forbs in the annual grassland.

The total litter from the previous year tended to be greater in areas "between bunches" in the restored perennial grassland, followed by annual grassland and near bunchgrass zones of the restored perennial grassland (Table 1). However, litter in the near bunchgrass zones stood largely upright and attached to the crown of the plant, rather than flattened on the ground as in the annual grassland and the annual-dominated areas in the between bunchgrass zones of the restored perennial grassland.

Root Distribution

Root biomass was generally similar between the annual and restored perennial grasslands and averaged about 0.8 g/kg dry soil for the whole profile (0–80 cm). For the four separate soil layers (0–15, 15–30, 30–60, and 60–80 cm) there were no significant differences in root dry weight (g/kg soil) between the three treatments with plant cover, partly due to large variation between samples in the same treatment (Fig. 2). Soil depth was the only factor affecting root biomass. A significant interaction, however, was observed for the factors treatment and depth, indicating different root distributions with depth in different treatments (Table 2). In annual grassland, more than 70% of the total root biomass occurred in the upper 15-cm layer, whereas perennial grassland samples tended to be more even in root biomass distribution with depth. The upper (0–15 cm) soil layer of the restored perennial plot contained only 40–50% of the total root biomass, whereas 30–40% was found in the 15- to 30-cm layer.

Root length in the annual grassland averaged about 100 m/kg dry soil (0- to 80-cm depth) compared to about 70 m/kg in both sampling zones of the perennial grassland, but the difference for the whole profile was not significant. In the uppermost layer (0–15 cm), however, root length of the annual grassland significantly exceeded that of the near bunchgrass zone in the perennial grassland by about 30–40% but was statistically similar to that of the between bunchgrass zone, which supported many of the same annual grass species. Like root biomass, root length had a more even distribution with depth in the perennial versus annual grassland. Samples from the near bunchgrass zone in the perennial grassland showed slightly higher values than the annual-dominated areas (Fig. 3). Again ANOVA resulted in a significant interaction for the factors treatment and depth (Table 2).

Table 1. Aboveground dry weight of plant species (g/m^2) and dead plant residues (litter) as sampled in 20 × 20–cm frames (average values, $n = 4$). Litter is dead material from the previous year.

	Treatment		
Plant Species	*Restored Perennial Grassland (near Bunches)*	*Restored Perennial Grassland (between Bunches)*	*Annual Grassland*
Grasses			
Aira caryophyllea (Aveneae tribe)	0.57	0.01	—
Bromus hordeaceus (Festuceae tribe)	17.60 a	24.47 a	50.46 b
Bromus madritensis (Festuceae tribe)	—	0.21	—
Bromus diandrus (Festuceae tribe)	2.39	0.43	1.20
Elymus glaucus[a,b] (Hordeae tribe)	18.27 b	— a	— a
Festuca spp. (Festuceae tribe)	3.99	5.50	7.71
Nassella pulchra[a,b] (Agrostideae tribe)	94.27 b	—a	— a
Unknown vegetative grasses	0.04	0.94	0.45
All grasses	137.12 b	31.55 a	59.82 a
Forbs			
Achyrachaena mollis[a] (Asteraceae)	—	—	0.54
Eremocarpus setigerus[a] (Euphorbiaceae)	—	—	0.04
Cerastium glomeratum (Caryophyllaceae)	— a	— a	2.28 b
Erodium cicutarium (Geraniaceae)	1.42 a	1.47 a	7.52 b
Galium spp. (Rubiaceae)	0.14	—	—
Gilia clivorum[a] (Polemoniaceae)	—	0.13	—
Hypochaeris glabra (Asteraceae)	0.57 b	— a	0.19 ab
Lupinus bicolor[a] (Fabaceae)	7.99	2.56	6.92
Lupinus nanus[a] (Fabaceae)	5.24	12.64	—
Phlox gracilis[a] (Polemoniaceae)	0.43	0.21	2.21
Plagiobothrys nothofulvus[a] (Boraginaceae)	—	—	0.28
Thysanocarpus curvipes[a] (Brassicaceae)	—	—	1.38
Trifolium bifidum[a] (Fabaceae)	—	0.07	—
Unknown vegetative forbs	0.04	0.73	—
All forbs	15.83	17.81	21.36
Sum (total biomass)	152.95 b	49.36 a	81.18 a
Total litter	115.41	153.49	122.74

The Tukey HSD test was applied for statistical groupings indicated by letters that differ from one another.
[a] Plant species native to the region.
[b] Plant species present in the seed mixture that was sown in the restored plots.

Chemical and Physical Properties of the Soil

Tillage significantly decreased the total soil C content in the surface layer (Table 3). The perennial grassland, last tilled 4 years before sampling, was intermediate between the tilled and the undisturbed annual grassland treatments. Total soil C generally decreased with increasing soil depth (Table 3). In all treatments, the amount in the 60- to 80-cm layer was about half that in the top layer, which ranged between 7 g C/kg dry soil (tilled bare soil) and 10 g C/kg dry soil (annual grassland). Soil C in the lower layers (deeper than 15 cm) was statistically similar in all four treatments. This was true for each layer separately as well as for the sum of the three layers. Total soil N showed a similar distribution. Depth and treatment were significant factors for both nutrients (Table 2). Higher C levels occurred on one side of the field, based on the significant block effect (Table 2). The average C-to-N ratio of all samples was 10.3 ± 1.1, and no significant differences in this ratio occurred among the field treatments or according to soil depth.

On an area basis, the untreated annual grassland showed the highest C storage down to 80 cm (6.2 kg C/m^2). By comparison, the tilled bare soil lost 1.5 (±0.4) kg C/m^2 in its 6 years without vegetation compared to the untreated grassland. The average values for the perennial grassland lay in between, with 5.2 kg C/m^2.

Bulk density increased slightly from about 1.4 to 1.5 g/cm^3 with soil depth (Table 3). Soil depth was the only factor affecting bulk density in the ANOVA (Table 2). No effect due to past or current tillage regime was detected. Even the recently tilled bare soil treatment showed no differences in the upper layer.

Soil Microbial Biomass and Soil Respiration

After 6 years without vegetation, the soil microbial biomass in the tilled plots decreased significantly from its starting point at approximately 215 (annual grassland) to 93 mg C/kg dry soil in the upper 15 cm of the soil profiles (Fig. 4). The microbial biomass in the restored perennial

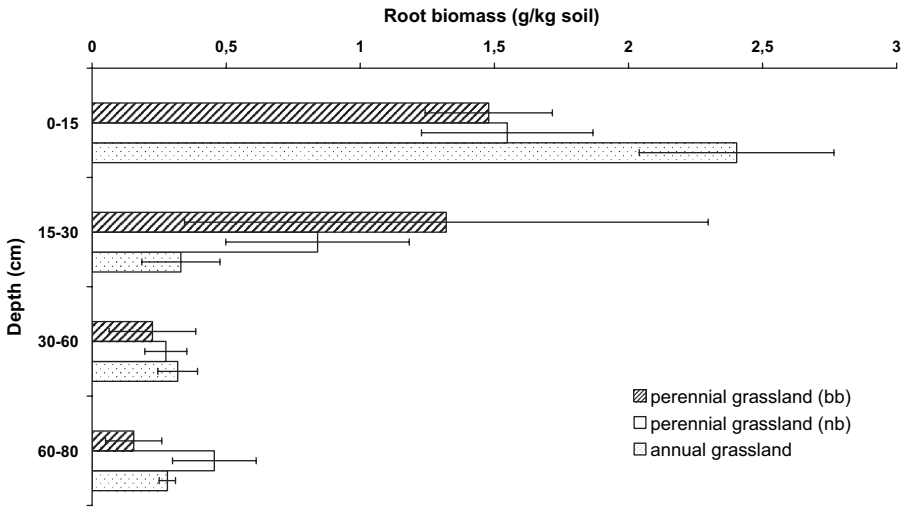

Figure 2. Root biomass in 0- to 80-cm soil profiles in annual grassland, restored perennial grassland, and tilled bare soil at Hastings Reserve in Carmel Valley, California. Restored perennial grassland is represented by two sampling zones, near the bunches (nb) and between the bunches (bb) (n = 4, standard error bars, statistical grouping by Tukey HSD).

grassland, however, either lost less C or regained C after the 2-year tillage episode and reached the same range as the untreated annual grassland in the top layer.

A strong depth gradient in microbial biomass occurred in the grasslands from the top layer to the 15- to 30-cm layer (Fig. 4). The tilled bare soil treatment had a more even distribution of microbial biomass C with depth and was not different from the grasslands below 15 cm depth. Microbial biomass C averaged about 70, 50, and 30 mg/kg dry soil in the 15- to 30-cm, the 30- to 60-cm, and the 60- to 80-cm layer, respectively, for all treatments. With the exception of the 60- to 80-cm layer in the restored perennial grassland (sampling zone near the bunches), which was significantly lower than the other treatments, no differences occurred between the grassland treatments for soil microbial biomass C.

Microbial biomass decreased from the top layer to the lower layers only in the plots with vegetation, which was demonstrated by a significant interaction between the factors treatment and depth (Table 2). As found for total soil C, soil microbial biomass showed a significant block effect but no interactions with treatment and depth. It had higher values but similar depth and treatment effects in the western block.

Soil respiration during 1-hr incubations averaged 3.2 mg/kg dry soil in the top layer of the annual grassland.

Table 2. Three-way ANOVA testing the effect of the factors treatment (T; n = 4), sample block (B; n = 2), and depth (D; n = 4) for soil samples (cf Fig. 1). No investigations on roots were carried out for the tilled plot.

Source of Variance		*T*	*B*	*D*	*T* × *B*	*T* × *D*	*D* × *B*	*T* × *B* × *D*
Root biomass	*F*	0.03	0.17	11.39	0.60	1.33	0.23	1.11
	P	n.s.	n.s.	***	n.s.	*	n.s.	n.s.
Root length	*F*	4.93	0.31	202.34	0.89	7.80	1.84	0.51
	P	*	n.s.	***	n.s.	***	n.s.	n.s.
Total soil C	*F*	8.08	17.87	75.03	1.55	1.67	0.60	0.90
	P	***	***	***	n.s.	n.s.	n.s.	n.s.
Total soil N	*F*	4.48	13.52	66.77	0.93	1.00	0.29	0.65
	P	**	***	***	n.s.	n.s.	n.s.	n.s.
Soil microbial biomass C	*F*	6.46	13.64	86.04	0.63	4.99	0.64	0.89
	P	**	***	***	n.s.	***	n.s.	n.s.
Soil respiration	*F*	8.08	0.61	56.36	0.44	1.70	1.54	0.98
	P	***	n.s.	***	n.s.	n.s.	n.s.	n.s.
CO_2 efflux	*F*	5.37	0.43		1.82			
	P	*	n.s.		n.s.			
CO_2 concentration in the soil	*F*	25.70	50.00	165.86	10.99	2.65	5.50	1.49
	P	***	***	***	***	*	**	n.s.
Bulk density	*F*	1.10	0.15	3.41	2.16	0.87	0.95	1.29
	P	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.

Data on CO_2 efflux were only tested for the factors T and B; n.s. = not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

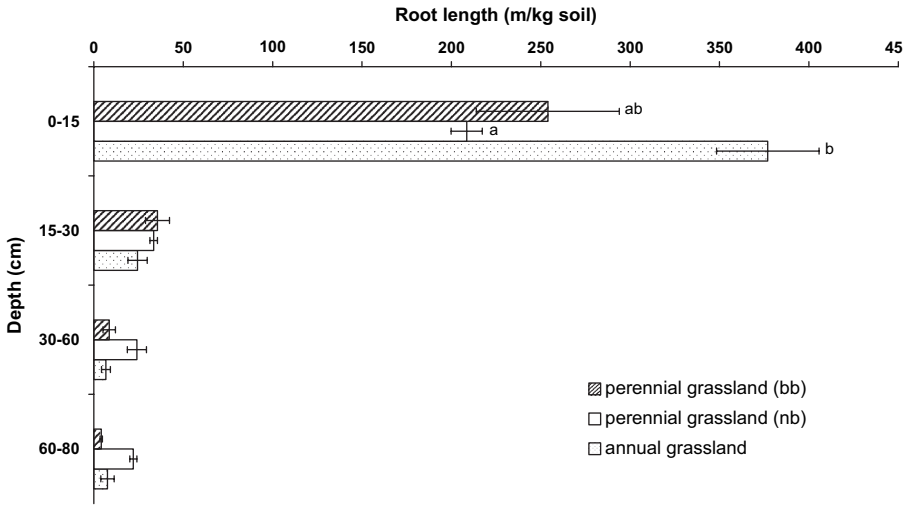

Figure 3. Root length in 0- to 80-cm soil profiles in annual grassland, restored perennial grassland, and tilled bare soil at Hastings Reserve in Carmel Valley, California. Restored perennial grassland is represented by two sampling zones, near the bunches (nb) and between the bunches (bb) (n = 4, standard error bars, statistical grouping by Tukey HSD).

The tilled bare soil treatment reached only 44% of this value and was significantly lower than the annual grassland (Table 3). The perennial grassland samples tended to have lower respiration rates in the upper layer than the annual grassland samples, but this reduction was not significant. Soil respiration was clearly a function of soil depth and treatment (Table 2).

CO_2 Emission and Concentrations in Different Soil Layers

CO_2 emissions ranged from 1.2 to 2.5 kg CO_2-C ha^{-1} hr^{-1} from the soil surface. No significant differences were found between the untreated annual grassland and either group of samples taken in the restored perennial grassland (Fig. 5). The tilled plot without vegetation showed a strong reduction in CO_2 emission. ANOVA showed a significant treatment but no block effect (Table 2).

The CO_2 concentration in the soil atmosphere increased in all plots with increasing soil depth (Fig. 6; Table 2), which was opposite of the depth distribution of soil C, soil microbial biomass C, and microbial respiration (Fig. 4; Table 3). CO_2 concentrations at all depths of the tilled, bare soil treatment were lower than in grassland plots with plant cover. Soil CO_2 concentrations were not different

Table 3. Total C and N, bulk density, and soil respiration for 0- to 80-cm soil profiles in annual grassland (a g), in restored perennial grassland (p g), and in tilled soil (t s) at Hastings Reserve in Carmel Valley, California. Perennial grassland was sampled near the bunches (nb) and in zones between the bunches (bb), where the plant cover consisted of annual grasses.

		Total Soil C (g/kg)			*Total Soil N*		*Bulk Density (g/cm^3)*		*Soil Respiration ($mg\ kg^{-1}\ hr^{-1}$)*		
Depth (cm)		*Mean*	*SD*	*sg*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*sg*
0–15	a g	9.88	0.22	b	0.90	0.08	1.41	0.03	3.20	0.74	b
	p g (bb)	8.50	1.09	ab	0.78	0.10	1.46	0.05	2.87	0.53	b
	p g (nb)	7.28	1.23	a	0.70	0.12	1.49	0.07	2.73	1.12	b
	t s	6.85	1.44	a	0.68	0.13	1.42	0.07	1.41	0.36	a
15–30	a g	6.65	1.07		0.60	0.08	1.34	0.11	1.51	0.43	
	p g (bb)	6.78	1.49		0.63	0.13	1.44	0.09	1.59	0.79	
	p g (nb)	6.55	1.76		0.60	0.16	1.46	0.04	1.97	0.78	
	t s	5.43	0.84		0.58	0.05	1.42	0.10	0.76	0.42	
30–60	a g	4.50	2.03		0.55	0.07	1.40	0.09	1.04	0.28	
	p g (bb)	3.48	0.57		0.43	0.06	1.46	0.13	0.66	0.22	
	p g (nb)	4.13	0.34		0.43	0.05	1.44	0.09	0.52	0.06	
	t s	3.93	1.07		0.43	0.06	1.49	0.14	0.55	0.17	
60–80	a g	4.83	1.67		0.45	0.21	1.54	0.07	0.42	0.24	
	p g (bb)	3.05	0.26		0.40	0.12	1.46	0.06	0.46	0.04	
	p g (nb)	3.33	0.54		0.40	0.08	1.50	0.12	0.41	0.18	
	t s	2.93	0.26		0.38	0.10	1.52	0.04	0.33	0.15	

n = 4, standard deviation (SD) and statistical grouping (sg) by Tukey HSD test, different letters indicate different groupings (only listed when differences were detected).

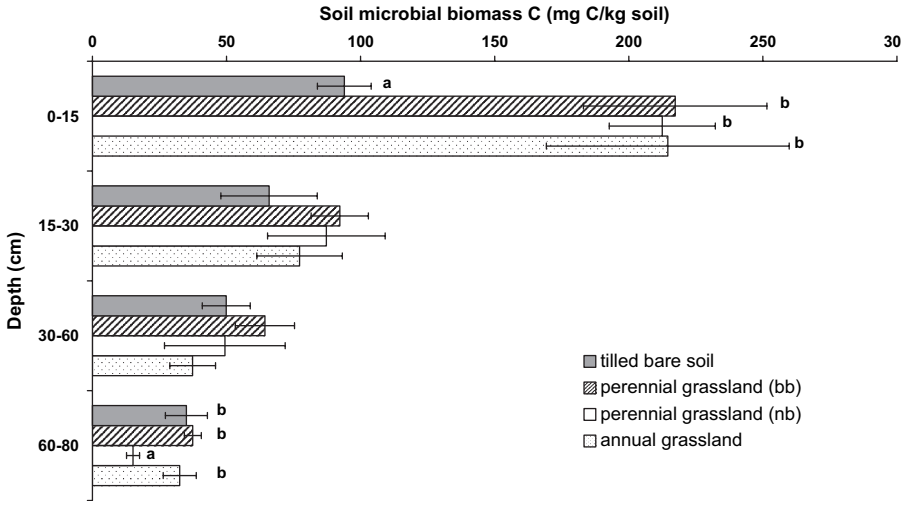

Figure 4. Soil microbial biomass C in 0- to 80-cm soil profiles in annual grassland, restored perennial grassland, and tilled bare soil at Hastings Reserve in Carmel Valley, California. Restored perennial grassland is represented by two sampling zones, near the bunches (nb) and between the bunches (bb) (n = 4, standard error bars, statistical grouping by Tukey HSD).

between annual grassland and the restored perennial grassland (Fig. 6).

Discussion

One of the few successful techniques for restoring native perennial grasslands in California is to sow seeds of perennial grasses into soil that has been tilled to reduce the dense stands of rapidly growing annual plants that outcompete native perennial seedlings (Stromberg & Griffin 1996). In this study, grassland restoration was accomplished by using tillage during the growing season for 2 years, application of herbicides, and harrowing the plot in close intervals before annuals set seed (Stromberg et al. 2002). Several impacts of this strong disturbance regime were found on the vegetation and soil chemical and soil microbial properties and functions. First, native perennial bunchgrasses were successfully established. The majority of the rest of the plant community made up a matrix of native and non-native annuals that represented a species composition quite similar to the surrounding annual grassland. Second, although tillage markedly reduced soil microbial biomass and respiration in the surface layer of soil (0–15 cm), levels were similar to those of annual grassland 4 years after native perennials were planted, indicating a rapid recovery. Third, the deeper (15–80 cm) distribution of total soil C and soil microbial C pools was

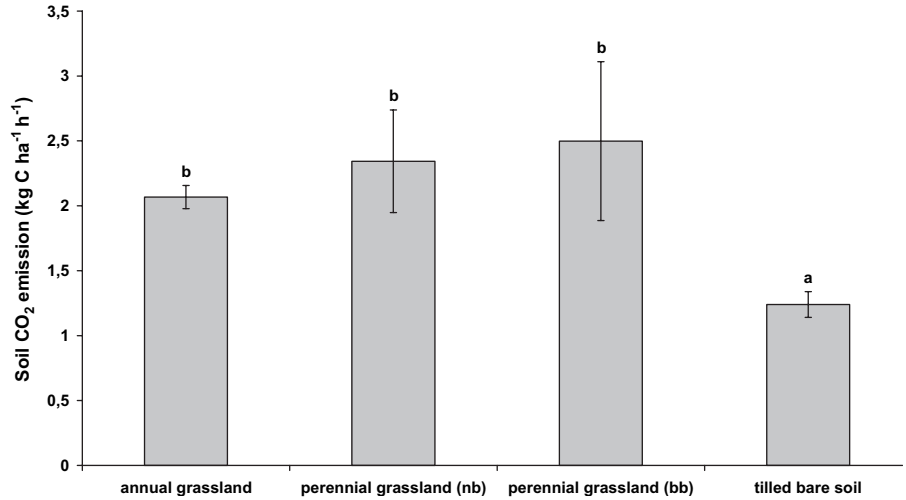

Figure 5. CO_2-C emission in annual grassland, restored perennial grassland, and tilled bare soil at Hastings Reserve in Carmel Valley, California. Restored perennial grassland is represented by two sampling zones, near the bunches (nb) and between the bunches (bb) (n = 4, standard error bars, statistical grouping by Tukey HSD).

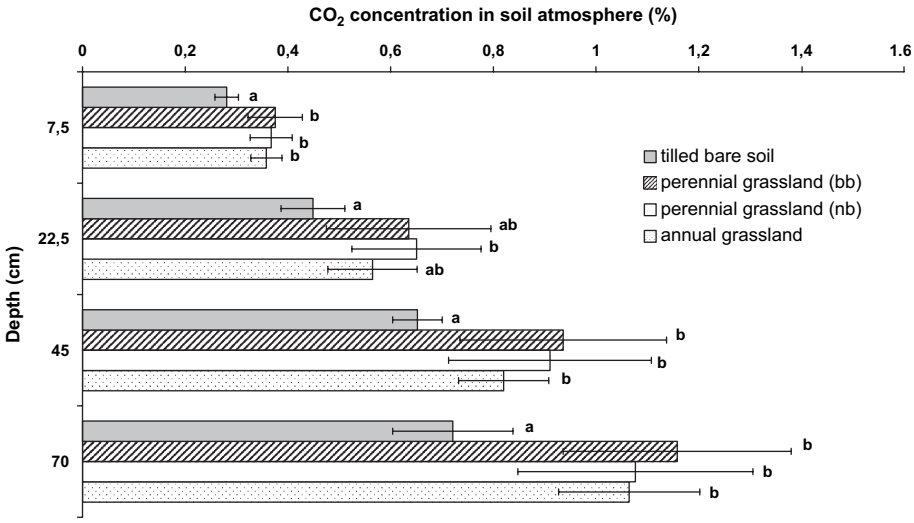

Figure 6. CO_2 concentrations in 0- to 80-cm soil profiles in annual grassland, restored perennial grassland, and tilled bare soil at Hastings Reserve in Carmel Valley, California. Restored perennial grassland is represented by two sampling zones, near the bunches (nb) and between the bunches (bb) (n = 4, standard error bars, statistical grouping by Tukey HSD).

only slightly affected by this method of grassland restoration and may increase through time based on the trend for more roots below 15 cm in the restored perennial compared to the annual grassland.

Vegetation

The plant community in the restored grassland plot contained a mixture of native and non-native annual forbs, non-native annual grasses, and native perennial bunchgrasses. Although species richness and composition was similar in the restored grassland and the annual grassland, the relative proportion of native plant species' biomass tended to be greater in the restored perennial grassland. In part, this was due to the high biomass of the seeded native bunchgrasses in the near bunchgrass areas as well as *Lupinus nanus* (Fabaceae) and *Lupinus bicolor* (Fabaceae) in between bunchgrass areas.

In addition to this analysis of vegetation from small areas above the sampled soil columns, cover was measured for the entire grassland areas at peak standing crop in 2001 and 2003 for a broader picture of species composition at the site. Note that plant cover exceeds 100% when the canopy of one species overlaps that of another species. Mean cover of perennial grasses for the 2 years was 9%, compared to 110% for annuals at the restored perennial site (Stromberg, unpublished data). Mean total cover in the annual grassland site was similar (120%). The restored perennial grassland had 14% mean cover of native annuals, compared to 10% in the annual grassland, and both grasslands supported similar numbers of native forbs (six to eight species sampled each year). Thus, the restored perennial grassland was dominated by between bunchgrass zones, and despite the history of disturbance, native forbs had become as important as in the annual grassland.

The higher aboveground biomass of natives near bunchgrasses, including the bunchgrasses *Nassella pulchra* and *Elymus glaucus*, than that of between bunchgrasses supports the idea that perennial grasslands promote above- and belowground spatial heterogeneity (Hook et al. 1991), whereas annual grasslands tend to have fairly continuous effects. Although differences in soil biological responses were not clearly observed among the three grassland sites in the time frame of this study, differences in litter quality between perennial and annual grasses (Hooper & Vitousek 1998) may increase soil spatial heterogeneity near older bunchgrasses (Hook et al. 1991). The effects of root tissue quality of perennial versus annual grasses in California grasslands on soil N fertility and C accumulation have yet to be definitively tested, but evidence suggests that soil C content and N immobilization may be enhanced by perennial bunchgrasses. Hooper and Vitousek (1998) observed lower N concentration in aboveground litter in *N. pulchra* than for annual grasses, but lignin content was also reduced so that lignin-to-N ratios were not significantly different from those of annual grasses. Because perennial grass litter remains attached to the bunchgrass crown and is still upright in the next growth season (L. E. Jackson, unpublished observation), the quality of the aboveground litter that reaches the soil surface may be much poorer than what is reported by Hooper and Vitousek (1998). Additionally, long-lived roots of perennial bunchgrasses may have lower N concentrations than those of the annuals. Lower root tissue quality of perennial bunchgrasses and the accumulation of soil C from perennial root deposition may increase potential N immobilization (Barrett & Burke 2000) in restored perennial

bunchgrass areas. Alternatively, higher labile root biomass in annual grasslands may also increase soil microbial biomass, thus increasing the potential for microbial N immobilization (Hooper & Vitousek 1998). Future sampling at this site may indicate whether such changes take place.

Given the differences in life form of the dominant plants in the annual and perennial grasslands, and the known ability for native perennial bunchgrasses to form deep root systems (Holmes & Rice 1996), established perennials in the restored site might have been expected to produce more roots at lower depths than annuals. Slightly deeper and more even root distribution near bunchgrasses now occurs in the recently restored perennial grassland compared to areas between bunchgrasses and annual grassland that both support annual grasses and forbs. Vertical stratification due to the presence of perennial bunchgrasses and development of their roots is expected to increase with time. This has been observed in older stands of relict perennial grasses and those grown in controlled environments (Holmes & Rice 1996).

Management Impacts on Soil Biota

Microbial biomass and activity in the restored perennial grassland approached the former conditions represented by the untreated control plots in the annual grassland, 4 years after the perennial seeds were sown in tilled soil. This similarity can be attributed to a regrowth of microbiota and microbial-related processes after cessation of tillage, introduction of a C supply from the perennial plants, and return of annual grasses to the spaces between the perennial bunches. In comparison, the tilled plots without vegetation showed strong reductions in size and activity of soil microbiota. For a former permanent pasture in winter-cold climate in New Zealand, Aslam et al. (1999) found a strong decline in microbial biomass caused by plow-based tillage within 2 years after the plot was taken into cultivation, but no-till management showed no changes in microbial biomass compared to the permanent pasture as the untreated reference.

Microbiota responded to tillage and the lack of vegetation in the tilled plots, indicating a low degree of resistance to the disturbance regime. The microorganisms seem to respond quickly and directly to disturbance. On a similar soil, microbial community structure changed within hours after tillage, as referred by phospholipid fatty acid (PLFA) profiles for a grassland soil, followed by community changes and a decline in the total amount of microbial biomass during the next week (Calderón et al. 2000). This process depends on the tillage practice applied; a stronger reduction of microbial biomass occurs for rototillage than for disking (Calderón & Jackson 2002). With respect to the results of Calderón et al. (2000) it is likely that the reduction and later regrowth of microbial biomass in the restoration process is accompanied by alterations in the community structure of microbiota and corresponding specific catabolic capabilities.

In addition to tillage, soil organisms were also undoubtedly affected by the lack of vegetation inputs. For example, cover crops in cultivated farmland can result in higher microbial biomass than bare soil fallows like our tilled plots (Mendes et al. 1999; Schutter & Dick 2002). For vegetable rotations in the nearby Salinas, California, area on the same type of soil, Jackson (2000) found that soil microbial biomass activity and N reutilization by following crops increased after cover crops.

Another aspect of land preparation in this study was the herbicide use in addition to tillage prior to seeding bunchgrasses. It is more likely that the application of glyphosate (Roundup) led to short-term increases in microbial biomass C than that the application had reductive effects. Haney et al. (2000) reported a constant size of microbial biomass after glyphosate application in a silt loam. However, they found a rapid and strong increase in C mineralization after application, indicating direct degradation and utilization of the applied compound by microbes. Other agents such as imazamox, benfluralin, or bensulfuron-methyl are toxic and can lead to different responses, such as rapid decreases in microbial biomass in soil (El-Ghamry et al. 2002; Vischetti et al. 2002).

Because effects of cultivation on soil microbial properties occur rapidly, much of the decline in microbial biomass in the surface layer may have occurred during the first 2 years of tillage. This was confirmed by 3-fold lower concentrations of total PLFA, as an alternative indicator for microbial biomass, in the tilled fallow plots than in annual grassland in the surface layer in spring 1998 (Steenwerth et al. 2003). By 2002, microbial biomass C, as measured by chloroform fumigation extraction, was only two times lower in the tilled plots, indicating some stabilization in the rate of decline of microbial biomass C, although the two methods may not be directly comparable. Interestingly, microbial biomass C was also about 100 μg/g soil at 0- to 15-cm depth in intensively tilled vegetable fields on closely related soil types in the Salinas Valley that also received little input of organic matter, suggesting a similar stabilization level as the bare soil in the tilled plots (Wyland et al. 1994; Calderón et al. 2000). Reduced microbial respiration, soil CO_2 concentration, and reduced surface CO_2 emission accompanied the decline in soil microbial biomass, indicating low labile and available C in the tilled plots. Declining microbial biomass in the surface layer is likely to be associated with reduced nutrient retention and soil fertility (Powlson et al. 1987), less soil aggregation and poorer soil structure, causing a less favorable root environment, and more susceptibility to erosion (Kay 1990).

Carbon Retention in the Soil Profile

Loss of total soil C, as expected, occurred more gradually through time than that of microbial biomass C. The losses of soil C in the tilled soil (24% of the annual grassland at 0- to 80-cm depth after 6 years) can be explained by the low total soil C amounts in the sandy loam soil and the high

frequency of physical disturbance by tillage. Tillage is known to stimulate net mineralization of organic C, in part due to changes in soil physical properties, such as lower bulk density and higher porosity, which increase soil temperature and thus microbial activity (Dao 1998; Silgram & Shepherd 1999; Calderón & Jackson 2002; Jackson et al. 2003).

CO_2 concentrations in the soil profile are indicative of microbial and root activity but are influenced by soil physical properties and diffusion processes. The significant reduction in the tilled plots for all soil layers cannot be simply attributed to microbial processes because microbial biomass and respiration were similar among treatments, except for the top layer (0–15 cm). This suggests that the CO_2 surplus in the lower layers of the plots with vegetation can be related to root respiration. The increase in CO_2 concentration with depth is expected because subsurface gas exchange rates decline with soil depth, leading to higher CO_2 saturations of soil atmosphere in lower layers, as long as oxygen and C sources enable C oxidation. Gas diffusivity in soil, which depends not only on pressure and temperature but also on the volume of air-filled pore space and on the shape of soil pores and their continuity (Glinski & Stepniewski 1985; Rolston & Moldrup 2002; Schwendenmann 2002), should be similar between the treatments below the tilled zone, based on similar bulk density and hydraulic conductivity (data not shown).

In the grassland restoration site, CO_2 surface efflux equaled that of the undisturbed annual grassland, whereas the tilled plots were significantly reduced to 75% of the CO_2 emission recorded for the annual grassland. Because C inputs to soil from plants are absent, assuming inputs from bacterial autotrophs and algae are negligible, the emissions from the tilled plot are net losses to the atmosphere, including more and older resistant C sources rather than fresh plant residues. This implies that periods with bare soil should be minimized in restoration practices to minimize the greenhouse effect from CO_2 emissions in global warming, decrease net losses of soil organic matter, and ultimately increase soil fertility.

In summary, the annual and the restored perennial grasslands after 6 years of land preparation and new stand establishment have similar soil microbial biomass, soil respiration, CO_2 emission, and also a similar distribution of CO_2 with depth. This indicates that soil biological properties are not strongly affected by plant community composition, at least at this stage in the restoration process. If differences in microbial community composition did occur, there appears to have been little effect on soil C pools and activity. Only the root distribution with depth responded slightly to the difference in grassland types. Over a longer period, turnover of perennial roots and production of root exudates may fuel microorganisms, possibly creating a gradual, slow process of increased microbial biomass and C storage in lower layers of Central Coast grasslands after restoration of native perennial bunchgrasses.

We conclude that

(1) The microbial biomass, which serves as a reservoir of C and an agent of nutrient turnover, is strongly affected by the cultivation procedures in the surface layer needed to deplete the annual seed bank in the restoration process.
(2) Highly manipulative restoration procedures that employ repeated tillage and herbicide applications appear worthwhile because the microbiota have a high resilience and can return to their former performance after bunchgrasses are established for several years on cultivated sites.
(3) Microbial biomass and activity seem to be mainly affected by aboveground litter deposition and processes in the surface (0–15 cm) soil layer. This suggests that added organic matter may promote greater soil microbial biomass in the early stages of restoration. Care should be taken, however, to avoid net microbial N immobilization because young bunchgrasses require N for growth.
(4) If the restoration goal is to increase soil C on a long-term scale, the system should be monitored over years to decades. In this study, microbial biomass and activity of lower soil layers remained stable and were little affected by plant species composition, aboveground alterations, or past disturbance, at least at this early stage of restoration.

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LITERATURE CITED

Aon, M. A., D. E. Sarena, J. L. Burgos, and S. Cortassa. 2001. (Micro)biological, chemical and physical properties of soils subjected to conventional or no-till management: an assessment of their quality status. Soil & Tillage Research **60:**173–186.

Aslam, T., M. A. Choudhary, and S. Saggar. 1999. Tillage impacts on soil microbial biomass C, N and P, earthworms and agronomy after two year of cropping following permanent pasture in New Zealand. Soil & Tillage Research **51:**103–111.

Barrett, J. E., and I. C. Burke. 2000. Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. Soil Biology & Biochemistry **32:**1707–1716.

Beck, T. 1984. Der Einfluss unterschiedlicher Bewirtschaftungsmassnahmen auf bodenmikrobiologische Eigenschaften und die Stabilität der organischen substanz in Böden. Kali-Briefe (Büntehof) **17:**331–340.

Beese, F., A. Hartmann, T. Beck, R. Rackwitz, and Z. Zelles. 1994. Microbial community structure and activity in agricultural soils under different management. Journal of Plant Nutrition and Soil Science **157:**187–195.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biology & Biochemistry **17:**837–842.

Burcham, L. T. 1957. California range land: an historico-ecological study of the range resource of California. California Division of Forestry, Department of Natural Resources, Sacramento, California.

Calderón, F. J., and L. E. Jackson. 2002. Rototillage, disking, and subsequent irrigation: effects on soil nitrogen dynamics, microbial biomass, and carbon dioxide efflux. Journal of Environmental Quality **31:**752–758.

Calderón, F. J., L. E. Jackson, K. M. Scow, and D. E. Rolston. 2000. Microbial responses to simulated tillage and in cultivated and uncultivated soils. Soil Biology & Biochemistry **32:**1547–1559.

Cione, N. K., P. E. Padgett, and E. B. Allen. 2002. Restoration of a native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in southern California. Restoration Ecology **10:**376–384.

Cook, T. D. 1978. Soil survey of Monterey County, California. United States. Department of Agriculture, Soil Conservation Service, U.S. Forest Service, and University of California Agricultural Experiment Station. Washington, D.C.

Dao, T. H. 1998. Tillage and crop residue effects on carbon dioxide evolution and carbon storage in a Paleustoll. Soil Science Society of America Journal **62:**250–256.

De Deyn, G. B., C. E. Raaijmakers, H. R. Zoomer, M. P. Berg, P. C. de Ruiter, H. A. Verhoef, T. M. Bezemer, and W. H. van der Putten. 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature (London) **422:**711–713.

Doran, J. W. 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Science Society of America Journal **44:**765–771.

Doran, J. W., and D. M. Linn. 1994. Microbial ecology of conservation management systems. Pages 1–27 in J. L. Hatfield, and B. A. Stewart, editors. Soil biology: effects on soil quality. CRC Press, Boca Raton, Florida.

El-Ghamry, A. M., J. M. Xu, C. Y. Huang, and J. Gan. 2002. Microbial response to bensulfuron–methyl treatment in soil. Journal of Agricultural and Food Chemistry **50:**136–139.

Follett, R. F., and D. S. Schimel. 1989. Effect of tillage practices on microbial biomass dynamics. Soil Science Society of America Journal **53:**1091–1096.

Francis, G. S., F. J. Tabley, and K. M. White. 2001. Soil degradation under cropping and its influence on wheat yield on a weakly structured New Zealand silt loam. Australian Journal of Soil Research **39:**291–305.

Glinski, J., and W. Stepniewski. 1985. Soil aeration and its role for plants. CRC Press, Inc., Boca Raton, Florida.

Haney, R. L., S. A. Senseman, F. M. Hons, and D. A. Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science **48:**89–93.

Heady, H. F. 1977. Valley grassland. Pages 491–514 in M. G. Barbour, and J. Major, editors. Terrestrial vegetation of California. John Wiley & Sons, New York.

Hickman, J. C. 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley.

Holmes, T. H., and K. J. Rice. 1996. Patterns of growth and soil-water utilization in some exotic annuals and native perennial bunchgrasses of California. Annals of Botany **78:**233–243.

Hook, P. B., I. C. Burke, and W. K. Lauenroth. 1991. Heterogeneity of soil and plant N and C associated with individual plants and openings in North American shortgrass steppe. Plant and Soil **138:**247–256.

Hooper, D. U., and P. M. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs **68:**121–149.

Huenneke, L. F. 1989. Distribution and regional patterns of California grasslands. Pages 1–12 in L.F. Huenneke, and H. A. Mooney, editors. Grassland structure and function: California annual grassland. Kluwer, Dordrecht, The Netherlands.

Jackson, L. E. 1985. Ecological origins of California's Mediterranean grasses. Journal of Biogeography **12:**349–361.

Jackson, L. E. 2000. Fates and losses of nitrogen from a nitrogen-15-labeled cover crop in an intensively managed vegetable system. Soil Science Society of America Journal **64:**1404–1412.

Jackson, L. E., F. J. Calderón, K. L. Steenwerth, K. M. Scow, and D. E. Rolston. 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma **114:**305–317.

Jenkinson, D. S. 1988. The determination of microbial biomass carbon and nitrogen in soil. Pages 368–386 in J. R. Wilson, editor. Advances in nitrogen cycling in agricultural ecosystems. CAB International, Wallingford, United Kingdom.

Jenkinson, D. S. 1990. The turnover of organic carbon and nitrogen in soil. Philosophical Transactions of the Royal Society, Series B **329:**361–368.

Joergensen, R. G. 1996. The fumigation extraction method to estimate soil microbial biomass: calibration of the k_{EC}-factor. Soil Biology & Biochemistry **28:**25–31.

Kay, B. T. 1990. Rates of change of soil structure under different cropping systems. Advances in Soil Science **12:**1–41.

Lynch, J. M., and E. Bragg. 1985. Microorganisms and soil aggregate stability. Advances in Soil Science **2:**134–170.

Mendes, I. C., A. K. Bandick, R. P. Dick, and P. J. Bottomley. 1999. Microbial biomass and activities in soil aggregates affected by winter cover crops. Soil Science Society of America Journal **63:**873–881.

Packard, S., and C. F. Mutel. 1996. The tallgrass restoration handbook for prairies, savannas, and woodlands. Island Press, Covelo, California.

Pella, E. 1990. Combustion gas analyzer method for total carbon and total nitrogen. American Laboratory **22:**16.

Potthoff, M., and F. Beese. 2000. Soil functions in the arable farming systems. Pages 81–108 in H. H. Steinmann, and B. Gerowitt, editors. Arable farming in a cultural landscape—functions and accomplishments. Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Research Centre Agriculture and Environment, Göttingen, Germany.

Powlson, D. S., P. C. Brookes, and B. T. Christensen. 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biology & Biochemistry **19:**159–164.

Rolston, D. E. 1986. Gas flux. Pages 1103–1119 in A. Klute, editor. Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd edition. Agronomy Monographs 9. Agronomy Society of America and Soil Science Society of America, Madison, Wisconsin.

Rolston, D. E., and P. Moldrup. 2002. Gas diffusivity. Pages 1113–1139 in J. H. Dane, and G. C. Topp, editors. Methods of soil analysis: Part 4. Physical methods. Soil Science Society of America, Madison, Wisconsin.

Schutter, M. E., and R. P. Dick. 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. Soil Science Society of America Journal **66:**142–153.

Schwendenmann, L. C. 2002. Below-ground carbon dynamics as a function of climate variability in undisturbed soils of a neotropical rain forest. Ph.D. thesis. University of Göttingen, Germany. Berichtsreihe des Forschungszentrums Waldökosysteme. Reihe A 184.

Silgram, M., and M. A. Shepherd. 1999. The effects of cultivation on soil nitrogen mineralization. Advances in Agronomy **65:**267–311.

Steenwerth, K. L., L. E. Jackson, F. J. Calderon, M. R. Stromberg, and K. M. Scow. 2003. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology & Biochemistry **35:**489–500.

Stromberg, M. R., and J. R. Griffin. 1996. Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. Ecological Applications **6:**1189–1211.

Stromberg, M. R., and P. Kephart. 1996. Restoring native grasses in California old fields. Restoration & Management Notes **14:**102–111.

Stromberg, M. R., P. Kephart, and M. Sicular-Mertens. 2002. Tillage: cheap weed and gopher control before planting native grasses. Grasslands **XII:**1, 6–7.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry **19:**703–707.

Vischetti, C., C. Casucci, and P. Perucci. 2002. Relationship between changes of soil microbial biomass content and imazamox and benfluralin degradation. Biology and Fertility of Soils **35:**13–17.

Walker, L. R., and R. del Moral. 2003. Primary succession and ecosystem rehabilitation. Cambridge University Press, Cambridge, United Kingdom.

White, K. L. 1967. Native bunchgrass (*Stipa pulchra*) on Hastings Reservation, California. Ecology **48:**337–357.

Woods, L. E. 1989. Active organic matter distribution in the surface 15 cm of undisturbed and cultivated soil. Biology and Fertility of Soils **8:**272–278.

Wu, J., R. G. Joergensen, B. Pommerening, R. Chaussod, and P. C. Brookes. 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biology & Biochemistry **22:**1167–1169.

Wyland, L. J., L. E. Jackson, and P. D. Brooks. 1994. Eliminating nitrate interference during Kjeldahl digestion of soil extracts for microbial biomass determination. Soil Science Society of America Journal **58:**357–360.

Zedler, J. B., ed. 2001. Handbook for restoring tidal wetlands. CRC Press, LLC., Boca Raton, Florida.