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Comfrey Mulch Enriches Soil, But Does Not Improve an Indicator Crop within One Season

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MMH, AAP, VK and KNJ designed the study. Authors MMH and AAP performed the experiment. Authors MMH, AAP, ARM, VK, ABG and DJB performed analyses. Authors MMH, VK, ABG, DJB and KNJ wrote the manuscript. All authors read and approved the final manuscript

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ABSTRACT

Comfrey (*Symphytum* spp.) is thought to accumulate plant nutrients such as potassium (K) in its leaves and is consequently used widely as a green mulch. We sought to investigate the efficacy of comfrey as a soil amendment by measuring its nutritional composition and the effects of mulching with comfrey on soil nutrients, soil microbial communities, and growth and quality of an indicator crop (kale) over one growing season in a small garden plot. We found that comfrey was rich in K and plots mulched with comfrey had higher concentrations of elemental K, as well as higher concentrations of available nitrogen, compared to plots mulched with paper. Diversity and composition of soil bacterial communities was similar between comfrey- and paper-mulched plots, but began to show a trend toward divergence by the end of the growing season. Overall, comfrey mulch did not enhance the yield or nutritional content of the kale, but perhaps could improve crop performance over a longer period of time or in K-limited soils.

Keywords: Symphytum; soil nutrients; mulch; microbial communities; Brassica oleracea; potassium.

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1. INTRODUCTION

Comfrey (*Symphytum* spp. and varieties, *Boraginaceae*) is widely grown as a companion plant or green mulch. This type of biological approach to nutrient management is part of many traditional agroecological methods, and the utilization of non-crop plants to serve support functions is integral to the systems approach of permaculture design [1]. With deep roots and a reputation for accumulating potassium (K) and other nutrients in its leaves [2] comfrey may serve as a "nutrient pump," increasing availability of some nutrients in topsoil, as has been demonstrated in other species [3,4]. This transdisciplinary study aims to track nutrients from comfrey leaf mulch to soil, and then to a fast-growing crop (kale, *Brassica oleracea* 'Red Russian'), in a test of how comfrey mulch might affect soil nutrients, soil microbial communities, and crop yield and quality.

For crops grown without synthetic fertilizers, pesticides, or regular irrigation, ability to cope with biotic and abiotic stresses is especially important for maintaining yield and quality. Ample K is critical for stress tolerance in plants [5,6] and plant-available forms often are deficient in agricultural soils around the world [7]. Organic mulches are an appealing approach to soil moisture and nutrient management, and frequently are recommended by organic gardening and permaculture practitioners [2, 8]. Supporting soil biological communities in turn affects soil properties such as texture and moisture as well as nutrient availability [9]. Whereas many benefits of organic mulches are well documented, the dynamics of nutrients such as K as they move from mulch to soil to microbial communities and plant roots are complex and likely to vary greatly under different growing conditions. Here we bring tools from several disciplines to address the effects of a widely used nutrient-rich mulch on soils, soil microbes, and crop plants. This study aimed to quantify nutrient inputs from an external source (mulch), the effect of these inputs on nutrient reservoirs in the soil, changes in soil microbial communities, and finally the effect on a crop and the removal of nutrients from the system at harvest (see concept map and summary of methods in Fig. 1).

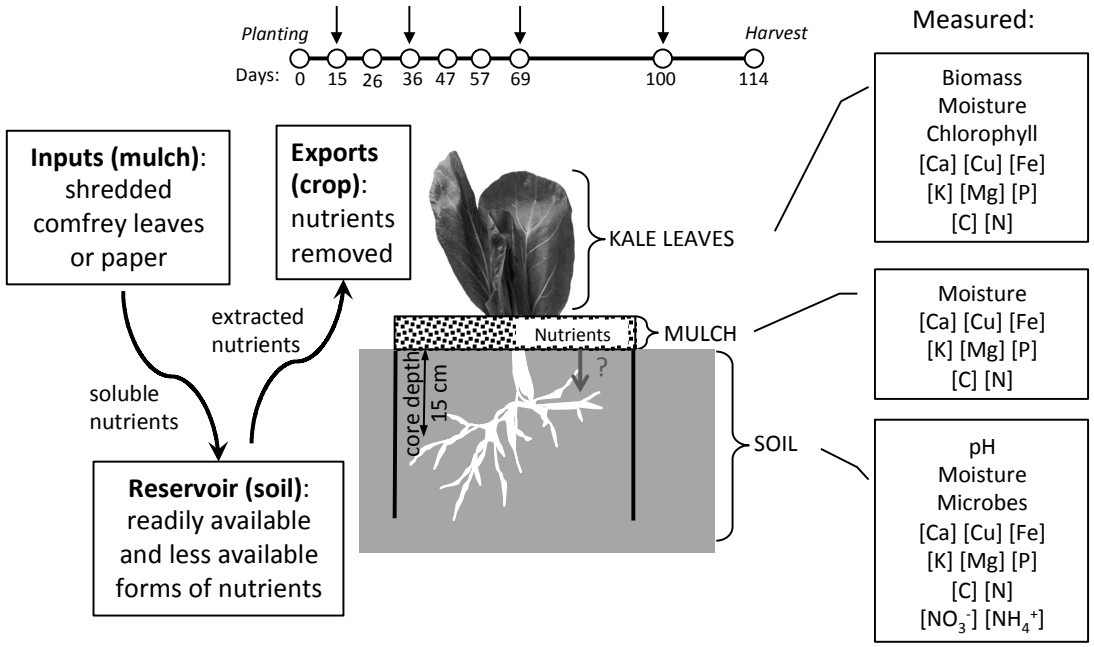

Fig. 1. Summary of methods and nutrient states measured

Kale plants in bottomless 28 cm diameter pots, were mulched with either shredded comfrey leaves or unbleached paper. Mulch was analyzed for nutrients. 15 cm soil cores were taken throughout the season and analyzed for pH, moisture, nutrients, and microbes. Chlorophyll was measured throughout the season in kale plants and at harvest leaves were analyzed for biomass, moisture, and nutrients. Circles on the timeline indicate data collection points; arrows indicate mulch applications.

2. MATERIALS AND METHODS

We quantified several macro and micronutrients in the three system reservoirs (mulch, soil, and crop tissue), due to the diverse ways nutrients move through the system and are processed in the soil. First we tested whether comfrey mulch was enriched in K and other essential nutrients, and then examined whether amending plots with comfrey affected the amounts of nutrients and composition of microbial communities in the soil relative to control plots mulched with shredded paper. Finally, because mulches can have complex effects on the greater agroecological community, for example by influencing herbivore populations [10] which in turn affect crop yield and quality, we assessed the integrated effects of the mulch by on an indicator crop plant (kale), measuring aboveground biomass, chlorophyll content, and concentration of K and other nutrients in leaf tissue of plants grown with and without comfrey mulch.

2.1 Plant Materials

Kale was used as an indicator crop in this study, as it has for other investigations of soil nutrient properties and additives [11]. Kale plants (*Brassica oleracea* Red Russian, Sustainable Seed Company, Covelo, California, USA) were grown from seed in a standard greenhouse. After 31 days (21 May 2014), ten were transplanted into 27 cm diameter individual plots defined by plastic bottomless pots filled with topsoil (25 cm deep) sunken into the ground in a common garden behind the Margaret Ferguson Greenhouses at Wellesley College (Wellesley, MA, USA, 42º17'39.0"N 71º18'07.9"W). Comfrey plants (*Symphytum* x *uplandicum* Bocking 14) were grown from root cuttings (Horizon Herbs, LLC, Williams, OR, USA) in an adjacent garden. First- and second-season comfrey plants were used as a leaf source for mulching. The kale plants were regularly irrigated with captured rainwater; comfrey plants only received water during rain, once established.

2.2 Mulch Treatments

Five randomly selected kale plants were mulched with shredded comfrey leaf mulch. The comfrey mulch was prepared by cutting fully expanded leaves into approximate 5 cm x 5 cm square pieces. Fresh comfrey mulch was initially applied 16 days after transplantation at a rate of 2.7 kg/m^2 and was replenished after 21, 53, and 81 days at rates of 2.7, 4.2, and 4.2 kg/m^2, respectively, based on wet mass. For comparison, the other 5 kale plants were mulched with unbleached paper (Scott hardroll towels 04142, Kimberly Clark, Franklin, MA, USA) shredded into 3 cm x 3 cm pieces, at a rate of 1.0 kg/m^2. Previous comparative studies have used shredded paper as a comparatively innocuous control mulch [12]. The mulch was contained in the plots via bird netting (15 mm mesh) secured with ground staples. Samples of both mulches were elementally analyzed using energy dispersive X-ray fluorescence (XEPOS, Spectro Analytical Instruments GmbH, Kleve, Germany) with a HOPG target to determine the nutrients (specifically Ca, Cu, Fe, K, Mg, and P) that they may add to the soil. In order to evaluate the reproducibility and accuracy of the XRF measurements NIST 2709a (San Joaquin soil) was measured six times and the average concentration for K (a representative cation) was 1.83 (SE 0.08) Wt% the accepted value 2.11 Wt%. Total C and N concentrations were also determined using an elemental analyzer (vario MICRO cube CHNS analyzer, Elementar, Ronkonkoma, NY, USA).

2.3 Assessment of the Yield and Quality of Kale Plants

In order to assess the effects of the different mulch treatments, the growth of the kale crops was measured throughout the season (0, 15, 26, 36, 47, 57, 69, 100, and 114 days post-transplantation) by measuring the height of the plants and the number of leaves. The quality of the plants was also approximated at the same interval by measuring the chlorophyll content index (CCI) of the 4th youngest leaf of each plant using a portable chlorophyll meter (CCM-200 plus, Opti-Sciences, Hudson, NH, USA). Total wet weight biomass was determined for all leaves at harvest (114 days post-transplantation). A sample of leaves from each plant was dried for 10 days at 45ºC to determine moisture content. The concentrations of Ca, Cu, Fe, K, Mg, and P, the soil nutrients reputedly accumulated by comfrey, were determined in dried and homogenized 4 g tissue samples (ground in a tungsten carbide mixer mill for 5 min) of the 10 youngest fully expanded leaves of each kale plant using XRF. Total C and N concentrations were measured in 2 mg dried leaf tissue samples using an elemental

analyzer. Overall nutrient accumulation values were estimated for each whole plant by extrapolating the dry weight crop biomass, based on measured moisture content, and multiplying by the known dry weight elemental concentrations.

2.4 Assessment of Soil Quality

In order to compare the effects of the mulch treatments on the soil, 15 cm deep soil cores were collected from each plot before planting and throughout the growing season at the same nine intervals at which kale measurements were taken. The soil cores were homogenized by mixing the soil in a 50 mL falcon tube by hand with a sterile spatula for 30 s and then vortexing the sample for 10 s, and then the subsamples for soil quality analysis were dried at 45℃ for 4 days. Soil pH was measured in a 1:1 soil:water slurry. Soil nitrate and ammonium concentrations were determined in extracts of 7 g dry soil and 40 ml 2 M KCl using the cadmium reduction and phenate methods [13] and a discrete analyzer (Astoria Discrete Analyzer, Astoria-Pacific International, Clackamas, OR, USA). At planting and harvest, soil Ca, Cu, Fe, K, Mg, and P concentrations were measured in 4 g soil samples, ground for 5 min in a mixer mill, via XRF. C and N concentrations were measured in 2 mg soil samples using an elemental analyzer. Concentrations of individual elemental soil nutrients, as well as changes in soil pH, were compared between mulch treatments at harvest using ANOVAs and concentrations of ammonium and nitrate were compared between mulch treatments over the growing season using repeated measures ANOVAs in JMP 11 [14].

2.5 Characterization of the Soil Microbial Communities

Out of the five planted plots per treatment, the bacterial community compositions of three randomly selected soil samples of each treatment at 0, 15, 26, 36, 69, 100, and 114 days post-transplantation, sampled and homogenized as described in section 2.4, were analyzed by 16S r RNA gene sequencing. DNA was extracted using the Power Soil® DNA Isolation Kit (MoBio, Carlsbad, CA) according to manufacturer's instructions and eluted in 50 µl of elution buffer. Once the DNA was extracted, it was quantified using Nano Drop (Thermo Scientific, Inc., Wilmington, DE, USA) and stored at –20ºC. All samples were sequenced at Forsyth Institute on a MiSeq Illumina platform (Illumina, San Diego, CA). DNA from each sample was amplified using the following universal 16S r RNA gene primers: 341F (5'-CCTACGGGAGGCAGCAG-3') and reverse 806R (5'-GGACTACHVGGGTWTCTAAT-3') with sequence adapters on both primers and sample-specific Golay barcodes on the reverse primer. The PCR amplicons were quantified by Pico Green (Invitrogen, Carlsbad, CA) using a plate reader. After quantification, amplicons were pooled in equal concentrations and the pool was cleaned up using Ultra Clean PCR Clean-Up Kit (Mo Bio, Carlsbad, CA). The pooled samples were sequenced on a MiSeq Illumina sequencer according to the sequencing procedures described in [15]. Raw sequence reads were submitted to NCBI Sequence Read Archive (SRA) under PRJNA354590.

Paired end reads were joined using Flash software [16]. Libraries were demulti plexed and filtered (phred q score ≥ 20) in MacQIIME v1.9.0 [15, 17]. Any reads that did not assemble by being perfectly matched in the overlapping region or meet the q-score threshold were discarded and were not used in subsequent analyses. Sequences were clustered using open-reference OTU picking approach at 97% similarity level using the Greengenes 2013 May 97% reference data set [18,19]. All samples were rarefied to 1300 sequences. Relative abundance plots, alpha and beta diversity metrics, UniFrac distances and PCoA plots were generated in QIIME [20] and visualized in R [21].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Nutrient content of mulches

In terms of essential plant nutrients, comfrey leaves were richest in K, with an elemental concentration of 63780 mg/kg (dry weight), 384-fold higher than the paper mulch (Table 1). Relative to paper, comfrey leaves were also rich in P, Fe, and Mg, with approximate 4-, 10-, and 6-fold higher elemental concentrations, respectively. The mulches contained similar concentrations of C, but the comfrey mulch was 40-fold richer in N. Paper mulch, on the other hand, contained more than twice as much Ca and Cu (Table 1).

3.1.2 Changes in soil quality

Reflective of the relatively high levels of K in comfrey mulch (Table 1), amendment with comfrey mulch resulted in a mean increase in soil K concentration of 1088 mg/kg over one growing season, whereas the soil in plots mulched with paper exhibited a mean decrease of 2144 mg/kg K (Table 2). While a significant difference in the change in soil K levels was observed between the two mulch types (ANOVA, $F_{1,8}$= 30.60, P<0.001), concentrations of the other nutrients reputedly accumulated by comfrey (Ca, Cu, Fe, Mg, and P) were not significantly enhanced in the comfrey-mulch plots relative to their paper counterparts. Comfrey leaves did not increase the total N in the soil (Table 2), despite their high N content (Table 1), perhaps not surprising given the dynamic nature of the soil N pool with multiple sinks. However, concentrations of both forms of plant available N, nitrate and ammonium, were significantly higher in comfrey-mulched plots than paper-mulched plots after the amendment was applied (Fig. 2, repeated measures ANOVAs for nitrate: $F_{1,8}$= 9.32, P = 0.016; for ammonium: $F_{1,8}$= 29.32, P<0.001). Mean (±SE) initial soil pH was 6.43 (±0.03) and there was a significant difference in pH change between the two mulch types over the growing season (ANOVA, $F_{1,8}$= 9.22, P= 0.016), as comfrey mulch alkalized the soil (mean(±SE) pH increase of 0.14(±0.06) and the paper-mulched soil became slightly more acidic (mean(±SE) pH decrease of 0.08 (±0.05). Soil moisture was similar between the treatments and ranged from 10-30% water content over all sampling points.

3.1.3 Soil microbial communities

There was a great diversity of taxa within each of the 34 soil samples for which the bacterial communities were analyzed, with no single dominant taxon (Fig. 3B). The 4 most dominant phyla recovered in the samples were *Proteobacteria* (44.3%), *Bacteroides* (13.4%), *Actinobacteria* (11.7%), and *Acidobacteria* (7.8%). Overall, there were 9 phyla that were present in at least one sample in abundance > 1%. At the family level, more than half of the community is composed of rare taxa. At the species level (97% sequence identity of 16S ribosomal RNA) there were 174 taxa present at 0.1% or greater. The most abundant taxon was only 4.6% of the community.

Soil microbial diversity was similar between the two mulch treatments. Nitrogen-fixing bacteria *(Rhizobiales - Hyphomicrobiaceae*) increased over time, but were not different between treatments. Principle coordinate analysis (PCoA) using weighted Unifrac values suggested that microbial communities each changed over the course of the 114 days of the experiment, and the communities began to show a trend toward divergence (Fig. 3A, PCoA unweighted - presence/absence data). Results from other recent studies have demonstrated shifts in microbial community composition and diversity with increases in soil nutrients [22].

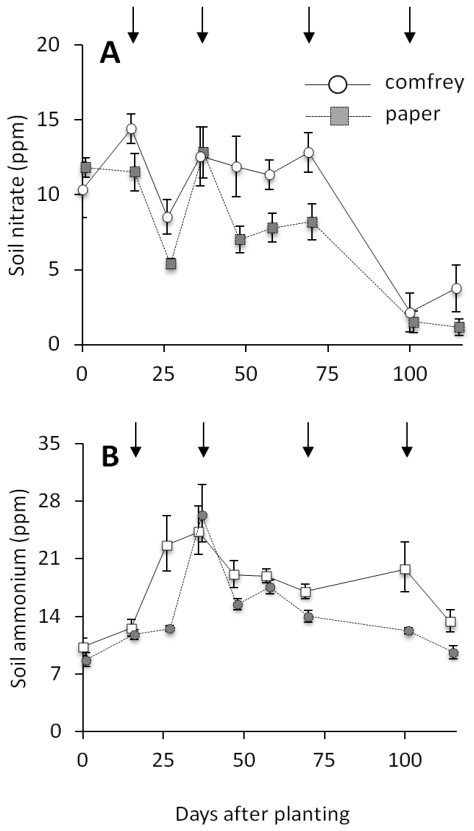

Fig. 2. Mean (± SE) soil nitrate (A) and ammonium (B) levels compared between plots mulched with comfrey and paper over a growing season.

Arrows indicate times at which comfrey mulch was applied/replenished. Ammonium means (± SE) are back-transformed from logarithmic data (n= 5).

3.1.4 Comparison of crops

The kale plants did not differ between comfrey- and paper-mulched plots in terms of leaf biomass, with each plant producing approximately 2 kg (wet weight). Furthermore, neither the chlorophyll content (data not shown) nor concentrations of the elements reputedly accumulated by comfrey differed between treatments in the kale leaves (Table 2).

3.2 Discussion

Despite large differences in elemental nutrient concentrations between the two mulches, and increased levels of K, nitrate, and ammonium in comfrey-mulched soils, mulch applications did not result in significant differences in the soil microbial communities ($P>0.05$; Wilcoxon test, Bonferroni adjusted p-value), nor in the size or nutrient quality of the indicator crop plants. While it is possible that a different crop that is more sensitive than kale to the availability of these nutrients might have responded more noticeably to the comfrey mulch, the mostly likely explanation for our results is that the existing topsoil was already sufficiently nutrient rich, as the kale grew vigorously in both treatments. If we were to repeat the experiment over several years in the same plots we might expect to observe a stronger effect of the comfrey amendment on crop yield, as the harvests remove nutrients from the system and the declining nutrient availability increasingly limits plant growth. Or, if we did not regularly irrigate the plants or otherwise allowed them to become more stressed, perhaps increased amounts of K in the soil would make a measurable difference [7].

Of course, the soil biological community plays a crucial role in these nutrient dynamics, so testing how the amendment affects this community is hugely important [22]. While our data suggested that microbial communities were beginning to diverge between the two treatments over the course of the experiment, further testing would be helpful. Sampling the top 15 cm of soil may have diluted mulch-based differences in both soil nutrients and microbial communities that most likely manifested most strongly in the upper layers of soil in direct contact with the mulch. However, our deep sampling reflects the microbial community that could potentially interact with a substantial proportion of the kale root system. It would be interesting to specifically examine the rhizosphere communities that colonize the roots of the kale plants between the mulch treatments to better assess the communities that might functionally interact with the plant and affect its phenotype, and to compare situations with different levels of water stress. A more complete analysis would also include fungi and other soil community members.

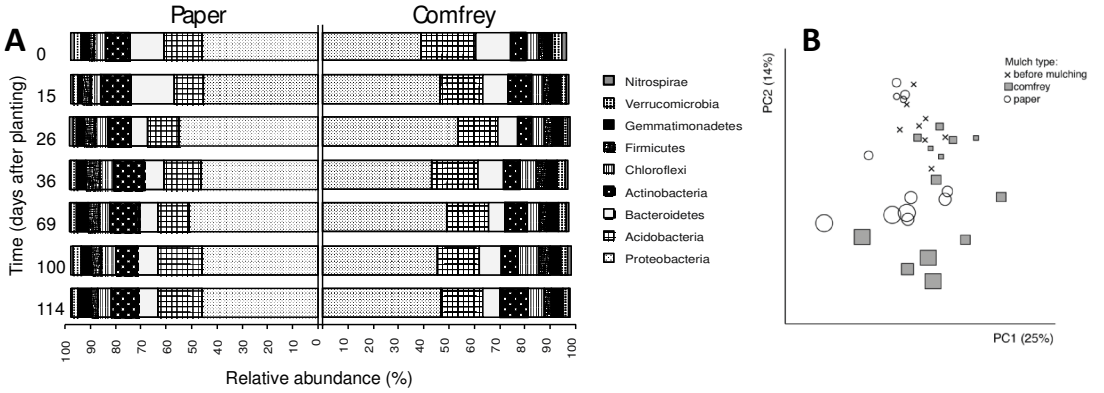

Fig. 3. Microbial succession viewed over the first 114 days post kale planting.

(A) Mean relative abundance of soil bacteria at the phylum level obtained from samples collected from three out of five randomly selected kale plants for each treatment. Only phyla with >1% mean relative abundance across all samples are shown. The mulch was applied post on days 16, 21, 53, and 81 post planting. (B) Principal coordinate analysis (PCoA) of weighted UniFrac distances of the soil microbiota at each sample point. PCoA of weighted UniFrac distances was performed to visualize differences in community structure at sequence depth of 2,200 sequences. Each point represents the microbiota in a single sample: control paper mulch (open circle), comfrey (gray square) and before mulching (black cross). All soil sample timepoints are displayed together and increase in size with time of sampling for days 26, 36, 69, 100, and 114 (n= 3).

Table 1. Comparison of elemental concentrations of nutrients in mulches and soil

	Ca	Cu	Fe	K	Mg	P	C	N
Concentrations in dried mulch (± analytical uncertainty), mg/kg								
Comfrey	14150 (±70)	14 (±1)	1060 (±10)	63780 (±130)	6120 (±10)	3330 (±80)	401800	4100
Paper	32157 (±90)	35 (±1)	170 (±20)	270 (± 60)	1690 (±150)	330 (±90)	417850	100
Total mass of nutrients added per plot, mg								
Comfrey	1590	2	120	7170	380	690	45170	3270
Paper	1840	2	10	20	100	20	23910	10
Initial soil concentrations (± SE), mg/kg	15800 (± 500)	25 (±1)	23000 (±1000)	18700 (±700)	2400 (±100)	4500 (±300)	44300 (±6600)	2300 (±300)

The total masses of nutrients added to the plots were calculated by multiplying the concentrations by the total amount of mulch added to the soil, including replenishments. Soil nutrient concentrations were measured in plots prior to mulch application (n= 5).

Table 2. Mean (± SE) elemental concentrations of nutrients in soil and kale leaves from plots amended with comfrey or paper

Element	Δ in soil concentration (mg/kg)		Kale leaf concentration (mg/kg)		Estimated amount in crop (mg)	
	Comfrey	**Paper**	**Comfrey**	**Paper**	**Comfrey**	**Paper**
Ca	-290 (±1030)	490 (±560)	10140 (±1040)	11030 (±950)	2630 (±220)	3340 (±840)
Cu	-1.5 (±1.4)	3.0 (±3.3)	6.3 (±0.9)	6.1 (±0.1)	1.6 (±0.1)	1.7 (±0.3)
Fe	-1420 (±560)	-270 (±1590)	73 (±8)	80 (±8)	19 (±2)	23 (±4)
K	**1090 (±280)**	**-2140 (±520)**	19200 (±2200)	17900 (±1300)	4960 (±390)	5330 (±1240)
Mg	400 (±270)	380 (±390)	4170 (±640)	3820 (±260)	1080 (±100)	1130 (±240)
P	460 (±70)	370 (±120)	4790 (±580)	4350 (±290)	1240 (±110)	1270 (±250)
C	11800 (±850)	3400 (±3000)	417500 (±3900)	414400 (±2500)	110000 (±12000)	11900 (±21000)
N	840 (± 440)	480 (±180)	35500 (±8200)	40000 (±4600)	9100 (±850)	11400 (±2400)

All concentrations are based on dry weights. Estimated amounts of elements in crop were calculated based on leaf concentrations and crop biomass values. Bolded values indicate significant differences between mulch treatments (ANOVA, $P \leq 0.05$, n= 5).

4. CONCLUSION

Our finding of increased levels of soil K, nitrate and ammonium in the comfrey treatment, compared to paper mulch, demonstrates that mulching with comfrey leaves can augment these critical nutrients in soils. When crop plants are stressed or these nutrients limit growth, it seems reasonable that comfrey mulch could improve the yield or quality of the crop. However, other nutrients that comfrey accumulates in its leaves, such as P, Fe, and Mg, were not significantly augmented in soils beneath comfrey mulch. The complexity of soil nutrient dynamics calls for bringing together perspectives and methods from biogeochemistry, microbiology, plant physiology and agroecology, as this project has attempted to do, to further our understanding and improve management of agricultural ecosystems.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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