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# Effects of Whole Orchard Recycling on Nitrate Leaching Potential in Almond Production Systems

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**Core ideas**

- Biomass recycling can mitigate nitrate discharges while conserving soil resources
- Biomass recycling immobilized fertilizer N without reducing leaching in the short-term
- Biomass recycling reduced nitrate leaching potential by 52% in the long term

## Abstract

Inefficient nitrogen (N) fertilization and irrigation have led to unhealthy nitrate levels in groundwater bodies of agricultural areas in California. Simultaneously, high commodity prices and drought have encouraged perennial crop growers to turnover less productive orchards, providing opportunities to recycle tree biomass *in situ* and use high carbon (C) residues to conserve soil and water resources. While climate change adaptation and mitigation benefits of high C soil amendments have been shown, uncertainties remain regarding the benefits and tradeoffs of this practice for N cycling and retention. We used established Almond [*Prunus dulcis* (Mill.) D. A. Webb] orchard trials on Hanford fine sandy loam with short-term and long-term biomass recycling legacies to better understand the changes in N dynamics and retention capacity associated with this practice. In a soil column experiment, labeled N fertilizer was added and traced into various N pools, including microbial biomass, and inorganic fractions in soil and leachate. Shifts in microbial communities were characterized using abundance of key N cycling functional genes regulating nitrification and denitrification processes. Our findings showed that, in the short-term, biomass recycling led to N immobilization within the orchard biomass incorporation depth zone (0-15 cm) without impacts on N leaching potential. However, this practice drastically reduced nitrate leaching potential by 52%, ten years after biomass incorporation, without increase in N immobilization. Although timing of these potential benefits as a function of microbial population and C and N biogeochemical cycles still need to be clarified, our results highlight the potential of this practice to meaningfully mitigate nitrate discharges into groundwater while conserving soil resources.

## Introduction

Nitrate is one of the most widespread contaminants of groundwater within agricultural regions, and therefore represents a threat to the quality and safety of drinking water (Bastani and Harter, 2019; van Grinsven et al., 2015). Intensive input-based agriculture and long-term use of synthetic N fertilizers and manures are the dominant non-point sources of N-based pollutants, primarily through nitrate leaching (Burow et al., 2008; Harter et al., 2017; Kourakos et al., 2012). This is exemplified in California, where inefficient nutrient management of irrigated specialty crop systems have turned one of the most productive agricultural regions in the world into a hotspot for nitrate contamination and low water quality (Harter et al., 2021; Lockhart et al., 2013). This contamination is especially pervasive in rural disadvantaged communities who rely solely on groundwater wells as potable drinking sources (Brown et al., 2013). Restoring water quality is therefore urgent and concerted efforts must be prioritized to reach the large reduction in nitrate discharge needed to maintain environmental integrity and ensure safe drinking water availability in an uncertain future.

Improving N management practices in perennial cropping systems has potential to meaningfully reduce nitrate leaching on a large scale as these systems are prominent and rapidly expanding across California's fragile watershed (CDFA, 2018; Khalsa and Brown, 2017; Zhang and Hiscock, 2016). In Almond production systems, high commodity prices and increasingly scarce irrigation water have promoted turnover of less productive orchards to new plantings and growing interest in using *in situ* biomass recycling as a climate adaptation and mitigation tool (Holtz et al., 2018; Jahanzad et al., 2020; Kendall et al., 2015). Whole orchard recycling refers to the grinding and soil incorporation of whole orchard biomass before replanting of a new orchard. It allows the addition of large quantities of C-rich woodchips to soils and has been shown to significantly increase soil C stocks and soils capacity to cycle nutrients and conserve water (Jahanzad et al., 2020; Kendall et al., 2015). Incorporation of high C:N residues also provides opportunities to retain N through recoupling of C and N biogeochemical cycles and improvements in soil health. While larger soil microbial communities may retain N in organic forms; improvements in soil physical characteristics and water retention capacity may further reduce losses associated with percolation and runoffs. The impacts of high C containing biomass on nitrate leaching have been mostly studied in the context of woodchips bioreactors to limit surface drainage in annual cropping systems (Christianson et al., 2012; Schipper et al., 2010; Warneke et al., 2011) but seldom documented when large amounts are incorporated *in situ* into agricultural soils upon replanting of orchards.

While soil health benefits associated with biomass recycling are documented in Almond systems (Holtz et al., 2016; Jahanzad et al., 2020), the short and longer-term impacts on nitrate leaching potential remain unclear. Studies point to reduced nitrate leaching with organic amendments through i) short term inhibition of organic N mineralization (Knowles et al., 2011) or N immobilization (Malcolm et al., 2019); ii) enhanced N retention due to improvements in soil physical and hydraulic properties, such as increased water holding capacity associated with within-aggregate soil pores (Insam and Merschak, 1997; Xu et al., 2016; Yoo et al., 2014) and iii) denitrification (Jang et al., 2019). Despite potential growth stunting with N immobilization during the first year after biomass incorporation, long term gains in soil health observed with whole orchard recycling highlight the potential of this practice to reduce nitrate discharges to groundwater and make significant improvements to the sustainability of perennial crop systems.

Understanding the mechanisms affecting N retention and cycling dynamics upon biomass recycling is critical to harness full potential of this practice to mitigate N losses to groundwater and develop efficient N fertilization strategies that minimize potential trade-offs associated with the application of high C containing orchard biomass. The objective of this study was to evaluate how soil N dynamics and retention were affected by biomass incorporation and its impacts on the fate of N fertilizer in the short (one year) and longer term (ten years). We hypothesized that large inputs of orchard biomass will mitigate fertilizer N leaching potential by i) rapidly increasing N cycling and net N immobilization through shifts in C pools and bacterial communities whereas ii) improved soil hydraulic characteristics associated with the addition of orchard biomass will drive changes in leaching potential on the longer term.

## Materials and Methods

**Site Description and Experimental Design**

Soil was collected in 2018 from two California almond orchard systems with short (one year) and long term (ten years) tree biomass management legacies before replant in Lincoln (short term, 36°38'37.0"N 119°30'37.7"W) and Kearney (long term, 36˚35'59.4"N 119˚30'11.7"W). Field sites are located nearby each other in a Mediterranean climate, with precipitation levels below evapotranspiration (ET) requirements during most of the growing season. In both sites, long term (68 years) annual rainfall and temperature averages are 285 mm and 17˚C, respectively. Soil at both locations are Hanford fine sandy loam (Supplementary Table S1). At the long-term experiment (Kearney), soil treatments were established in 2008 following termination and shredding of a 20-year-old peach (*Prunus persica* Var. Fay Elberta) orchard in a complete randomized design with seven replications. At the main plot level, two biomass management treatments were established: a whole orchard recycling treatment (+biomass), where the woody biomass was incorporated within the top 15 cm of soil in the tree row prior to planting by using a land clearing equipment (Ironwolf 700B Slasher, Nobe, OK, USA), which produced variable sizes of woody biomass ranging from ~ 5 cm to 30 cm and a control treatment (-biomass), where trees were uprooted, burnt, and ashes re-incorporated to the surface soil. Details of the trial design, establishment and management can be found in Jahanzad et al. (2020). At the short-term experiment (Lincoln), soil treatments were established in 2017 after termination of a 20-year-old Westerner plum (*Prunus salicina* Lind) orchard in a complete randomized design with four replications. Plots (60 × 33.5 m) were randomly established across the orchard upon replanting of almonds with either woody biomass incorporated within top 0-15 cm of soil (+biomass) or exported (-biomass).

**Sampling and Soil Column Experiment**

Undisturbed soil cores were taken in July 2018 for each site and biomass treatment (+/-biomass) from the berms between trees to a depth of 0-30 cm using an AMS (AMS Inc., American Falls, ID) soil core sampler with plastic storage liners. Four soil samples per treatments were taken from three randomly chosen replicates at each experiment (n= 24 soil cores from each experiment).

Four additional soil cores were sampled at the same locations for analysis of soil chemical properties, split into two soil depths (0-15 cm and 15-30 cm), composited for each depth zone and dried in a forced-air oven at 50˚C prior to analysis.

Undisturbed soil cores in the plastic-cylinder liners were directly set up as soil columns inside opaque PVC tubes to prevent soil disturbance from repacking and to maintain soil structural and hydrological properties associated with biomass inputs. Columns were secured in upright position using retort stands and rings and saturated with DI water (Mailapalli and Thompson, 2012). Excess water was allowed to drain prior to installing the soil columns on the retort stands to homogenize soil moisture at field capacity. Small holes were made in the bottom cap, which was filled with gravel, to allow percolation of the leachate without losing soil from the column. Leachate collection units (250 mL plastic container) were attached to the lower caps.

Isotopically labelled ammonium sulfate fertilizer [$(^{15}NH_4)_2SO_4$ 10% $^{15}N$] was dissolved with DI water and 10 μg N per g of soil were applied to the columns using burettes installed on top of the columns to allow a gradual matrix flow (Regehr et al., 2015). This ammonium-based fertilizer was chosen since it is one of the most common forms of inorganic N fertilizers applied to almond orchards and provides sufficient N and readily available sulfur to support plant growth. After 24 h (t=0), half of the soil columns were extracted and processed upon removal to allow for initial and complete immobilization of $^{15}N$ (Hood et al., 2003). The remaining soil columns were extracted and processed after 96 h (t=1), allowing sufficient time for N transformations to occur but before re-mineralization begins (Regehr et al., 2015). Soil columns were sliced using a hack saw to separate the 0-15 and 15-30 cm soil layers and subsamples were taken from each soil depth for analysis.

**Microbial Biomass N and Isotope Signature**

Microbial Biomass N (MBN) was measured on 6g of moist soil using the chloroform fumigation extraction method (Horwath and Paul, 1994). Dissolved N in extracts were measured using the alkaline persulfate oxidation method (Cabrera and Beare, 1993). Microbial biomass N was calculated by dividing the difference in N content between the fumigated and unfumigated samples using a 0.68 correction factor to account for incomplete N extraction (Horwath and Paul, 1994). The second set of soil samples were extracted with 0.25 M $K_2SO_4$ and liquid samples were then oven-dried (50$^o$ C) into a fine powder using a ball mill grinder and encapsulated into tin (Sn) capsules for $^{15}$N isotope analysis. An Elementar Vario EL Cube (Elementar Analysensysteme GmbH, Hanau, Germany) (Coyle et al., 2009; Stark and Hart, 1996) linked with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) was used at the University of California (UC) Davis Stable Isotope Facility for $^{15}$N isotope analysis.

Isotope composition of microbial biomass ($\delta^{15}N_{MB}$) was calculated using mass balance (Coyle et al., 2009) as:

$$\delta^{15}N_{MB} = \frac{([\delta^{15}N_F \times N_F]) - ([\delta^{15}N_{NF} \times N_{NF}])}{MBN}$$

where F and NF are fumigated and non-fumigated samples respectively, and MBN stands for microbial biomass N.

**Nitrogen Immobilization and Mineralization Rates**

The $^{15}$N isotope pool dilution technique was used to quantify gross N immobilization (GNI) and gross nitrogen mineralization (GNM) rates (Davidson et al., 1991). Briefly, N from 20g of moist soil was extracted with 100 mL of 2M KCl. Extracts were immediately frozen and analyzed at the UC Davis Stable Isotope Facility for ammonium content (Hannon and Bohlke, 2008). Gross N mineralization and immobilization rates were calculated as follows (Regehr et al., 2015):

$$m = \frac{[NH_4^+]_0 - [NH_4^+]_t}{\Delta t} \times \frac{\ln(APE_0/APE_t)}{\ln[NH_4^+]_0/[NH_4^+]_t}$$

$$i = \frac{[NH_4^+]_0 - [NH_4^+]_t}{\Delta t} \times \frac{\ln\left([NH_4^+]_0 APE_0\right)/\ln\left([NH_4^+]_t APE_t\right)}{\ln[NH_4^+]_0/[NH_4^+]_t}$$

where *m* is the gross N mineralization rate (μg N $g^{-1}$ soil $d^{-1}$) and *i* is the gross N immobilization rate (μg N $g^{-1}$ soil $d^{-1}$). $NH_4^+$ is the total soil ammonium content (μg N $g^{-1}$ soil); $\Delta t$ is the related time interval (days); *APE* is the atom percent $^{15}$N excess of $NH_4^+$; 0 (t=0) and t (t=1) indicate the two sampling time points. The net rate of immobilization (NRI) was calculated by subtracting the gross rate of mineralization (GNM) from the gross rate of immobilization (GNI) (Regehr et al., 2015).

**Nitrate Leaching**

Collected leachate samples from the soil columns were filtered with 0.2-micron filter upon collection and stored frozen. Samples were then analyzed for $^{15}N$ nitrate using bacterial denitrification assay using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany) (Rock and Ellert, 2007) at the UC Davis Stable Isotope Facility.

**Abundance of N Cycling Functional Genes**

Abundance of a subset of microbial genes involved in N-cycling processes was measured by Real-time PCR on DNA extracted from 0.25 g of soil subsamples using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Irvine, CA, USA). Nitrogen cycling functional genes involved in nitrification (bacterial *amoA*, *amoA*-1F and *amoA*-2R, Rotthauwe et al., 1997) and denitrification, including the copper-dependent nitrite reductase (*nirK*, F1aCu and R3Cu, Throbäck et al., 2004) and cytochrome $cd_1$-containing nitrite reductase (*nirS*, cd3aF and R3cd, Throbäck et al., 2004) were quantified by quantitative PCR (qPCR) using 10 uL reactions and the Stratagene Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent, Santa Clara, CA) as described in Schmidt et al. (2020) at the United States Department of Agriculture- Agricultural Research Service's (USDA-ARS) Crops Pathology and Genetics Research Center, Davis, CA.

**Soil Chemical Analysis**

Dry soil samples were analyzed at the UC Division of Agriculture and Natural Resources (UCANR) Analytical Laboratory. The pH was determined using a saturated paste method (Staff, 1954). Electrical conductivity (EC) was measured according to the method described by Rhoades (1982). Sodium (Na), Calcium (Ca), and Magnesium (Mg) were measured using Inductively Coupled Plasma Emission Spectroscopy (ICP-AES) (Meyer and Keliher, 1992), which followed a nitric acid/hydrogen peroxide microwave digestion method (Sah and Miller, 1992). Cation exchange capacity (CEC) was determined based on the method described by Rible and Quick (1960). Total N was measured by combustion method (ECS 4010, Costech Analytical Technologies Inc, Valencia, CA; USA). Soil organic matter (SOM) was measured using the Loss-On-Ignition Method (Nelson and Sommers, 1996).

**Statistical Analysis**

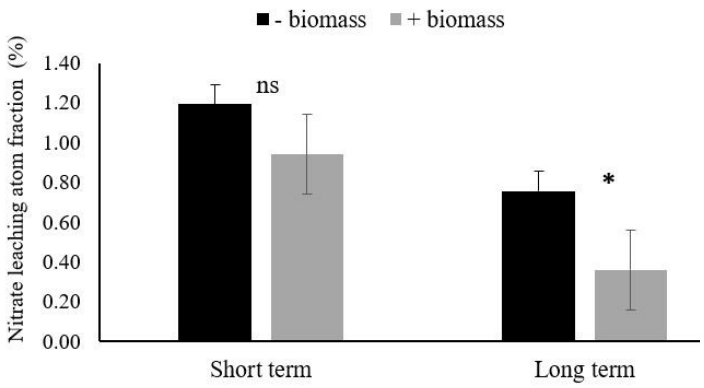
Data were analyzed using the PROC Mixed procedure with Kenward–Roger degrees of freedom approximation in SAS (SAS Institute, 2009).Soil treatments (+/- biomass) and soil depth and their interactions were considered as fixed effects, and blocks and the interaction of blocks with fixed effects as random effects. Data from the long-term and short-term sites were analyzed as separate experiments due to management practices differences and the variable timeframes of the experimental setups at the two distinct locations. The assumptions of ANOVA were tested, and transformations were applied where necessary to achieve normality and heterogeneity of residuals (i.e., *nirS*). When ANOVA showed significant fixed effects or interactions ($P < 0.05$), comparisons of means were made using the adjusted Tukey's range test.

## Results

**Nitrate Leaching**

Addition of woody biomass did not significantly mitigate leaching potential of fertilizer N on the short term (one year after addition) despite trends towards lower nitrate leaching losses in the +biomass treatment (1.19 vs 0.94, atom % $^{15}N$ for +biomass vs -biomass treatments) (Figure 1). Ten years after recycling, nitrate leaching from fertilizer N was significantly reduced by 52% compared to the -biomass treatment.

Figure 1. $^{15}NO_3^-$ recovered in leachate samples as affected by biomass incorporation at the long-term (10 years, Kearney) and short-term (1 year, Lincoln) experiments.




$^{ns}$ no significant difference; *significant difference at $P \leq 0.05$

**Gross N Mineralization**

We found no significant effects of biomass addition on GNM rates, either on the short or long term; with trends towards lower mineralization ten years after biomass incorporation. We observed higher GNM rate at lower soil layer (15-30 cm) compared to the topsoil (1.64 vs 1.02 $\mu gNg^{-1}$ soil $d^{-1}$) for both biomass treatments at the short-term experiment (Table 1).

**Microbial Biomass N and Isotope Signature**

More N was incorporated into microbial biomass (MBN) in the 0-15 cm soil layer compared to the 15-30 cm across both biomass treatments at the short-term experiment (+23% in 0-15 cm). Addition of woody biomass (+biomass) increased MBN by 95% compared to the -biomass control in the short-term across all soil depth (Table 1). Similarly, $^{15}N$ recovery of N fertilizer into microbial biomass ($\delta^{15}N_{MB}$) was 94% higher in the +biomass treatment than the -biomass, one year after incorporation, especially in the topsoil layer (Table 1).-No significant differences were found ten years post incorporation in terms of MBN and $\delta^{15}N_{MB}$, despite slight increases in the 0-15 cm soil layer with biomass addition (Table 1).

**Nitrogen Immobilization Rates**

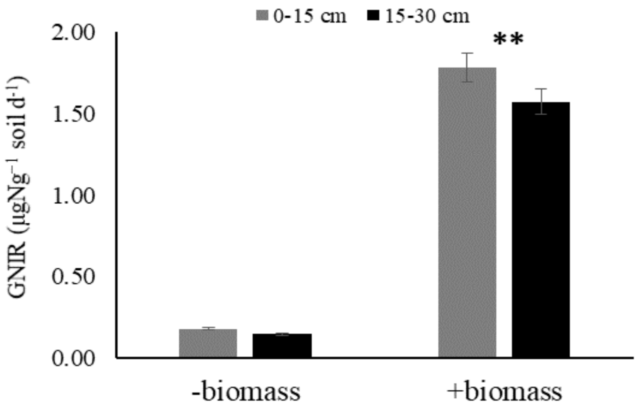
Soils in the recycled orchards demonstrated higher Gross N Immobilization (GNI) rates at the short-term experiment with a tenfold increase compared to the -biomass treatment across soil depths (1.67 vs 0.16 μg N $g^{-1}$ soil $d^{-1}$) (Table 1). There was a significant interaction of soil depth × biomass treatment with higher GNI rate observed in the 0-15 cm soil layer of +biomass soil compared to the lower soil depth (1.78 vs 1.57 μgN$g^{-1}$ soil $d^{-1}$, respectively; Figure 2). We also observed higher Net Rate of Immobilization (NRI) with biomass incorporation on the short term (0.65 vs -1.72 μgN$g^{-1}$ soil $d^{-1}$, respectively), especially in the topsoil layer. Although biomass addition did not significantly impact GNI at the long-term experiment, increases in NRI were observed with biomass addition across soil depths (Table 1).

Table 1. Impact of biomass inputs on microbial biomass nitrogen (MBN), isotopic signature of the microbial biomass ($\delta^{15}N_{MB}$), gross nitrogen immobilization (GNI) rate, gross nitrogen mineralization (GNM) rate, and net rate of immobilization (NRI) at two soil depths.

| Experiment | Treatment | MBN (μgN$g^{-1}$ soil $d^{-1}$) | $\delta^{15}N_{MB}$ (‰) | GNM | GNI | NRI |
|---|---|---|---|---|---|---|
| | | | | μgN $g^{-1}$ soil $d^{-1}$ | | |
| Short term | -biomass | 11.962 | 6.264 | 1.188 | 0.164 | -1.725 |
| | +biomass | 23.412 | 12.165 | 1.015 | 1.674 | 0.658 |
| | 0-15 cm | 19.573 | 11.962 | 1.026 | 0.979 | -0.280 |
| | 15-30 cm | 15.800 | 6.467 | 1.644 | 0.859 | -0.785 |
| Effects | ……p value…… | | | | | |
| | Biomass | 0.013 | 0.059 | 0.391 | 0.005 | 0.012 |
| | Depth | 0.022 | 0.044 | 0.027 | 0.557 | 0.052 |
| | Biomass × Depth | 0.269 | 0.308 | 0.894 | 0.033[¥] | 0.561 |
| Long term | -biomass | 13.477 | 6.446 | 4.061 | 0.557 | -3.904 |
| | +biomass | 13.944 | 7.676 | 2.665 | 0.832 | -1.835 |
| | 0-15 cm | 14.806 | 7.431 | 2.977 | 0.563 | -2.665 |
| | 15-30 cm | 12.615 | 6.691 | 3.755 | 0.426 | -3.074 |
| Effects | ……p value…… | | | | | |
| | Biomass | 0.867 | 0.241 | 0.062 | 0.681 | 0.041 |
| | Depth | 0.388 | 0.337 | 0.653 | 0.471 | 0.586 |
| | Biomass × Depth | 0.398 | 0.629 | 0.306 | 0.593 | 0.320 |

[¥] Refer to figure 2 for this significant interaction effect with depth.

Figure 2. Impacts of biomass incorporation on gross nitrogen immobilization (GNI) rate at different soil depths in the short-term experiment.




** significant difference at *P ≤0.01*

**Bacterial Functional N Cycling Genes**

Biomass inputs and soil depth significantly impacted the abundance of bacterial nitrification gene *amoA*; the most abundant gene involved in N cycling. The relative abundance of bacterial *amoA* genes was nine-fold higher in the 15-30 cm soil layer compared to the 0-15 cm layer (Table 2) with the highest abundance of bacterial *amoA* observed in the lower soil layer of the -biomass treatment (Figure 3). Albeit non-significant, the abundance of bacterial *amoA* tended to be greater in the +biomass treatment compared to the -biomass treatment across depth zones. Biomass incorporation led to lower abundance of *nirK* and the topsoil layer was richer in *nirK* functional genes (Table 2). No significant effect of soil depth or biomass treatments was observed on *nirS* abundance (Table 2).

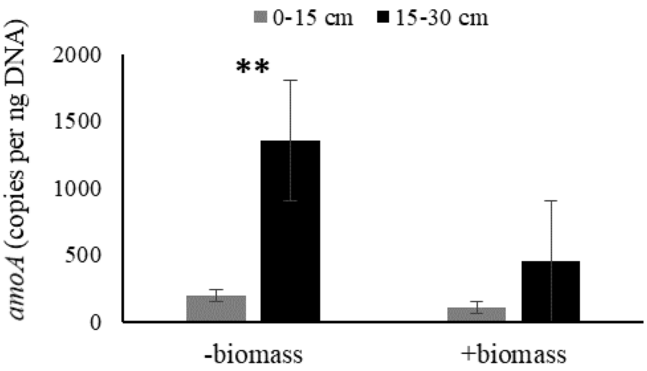
Soils from the long-term experiment showed tenfold greater abundance of *amoA* in the 15-30 cm soil layer compared to the 0-15 cm across biomass treatments (1025 vs 87 copies per ng DNA, respectively) (Table 2). Soils from the long-term experiment exhibited a 71% increase in abundance of nitrate reductase *nirK* with biomass incorporation (Table 2). Also, greater abundance of *nirK* was observed in the lower soil layer (15-30 cm) compared to the topsoil (8129 vs 4413 copies per ng DNA, respectively; table 2). Significantly higher nitrate reductase gene *nirS* was also detected in the lower soil depth compared to topsoil layer (3885 vs 529 copies per ng DNA, respectively) without significant impacts of biomass addition (Table 2).

Table 2. Impact of biomass inputs on abundance of bacterial *amoA*, *nirK*, and *nirS* at two soil depths.

| Experiment | Treatment | *amoA* | *nirK* | *nirS* |
|---|---|---|---|---|
| | | ………… copies per ng DNA……. | | |
| Short term | -biomass | 279 | 40525 | 1003 |
| | +biomass | 773 | 26125 | 1295 |
| | 0-15 cm | 147 | 37311 | 992 |
| | 15-30 cm | 905 | 29339 | 1306 |
| Effects | ………………..………………..……p value…..…..……... | | | |
| | Biomass | 0.071 | 0.022 | 0.499 |
| | Depth | <.0001 | 0.034 | 0.384 |
| | Biomass × Depth | 0.008[¥] | 0.220 | 0.841 |
| Long term | -biomass | 545 | 4572 | 2427 |
| | +biomass | 567 | 7969 | 1986 |
| | 0-15 cm | 87 | 4413 | 529 |
| | 15-30 cm | 1025 | 8129 | 3885 |
| Effects | ………………..………………..……p value…..…..……... | | | |
| | Biomass | 0.941 | 0.038 | 0.400 |
| | Depth | <.0001 | 0.023 | <.0001 |
| | Biomass × Depth | 0.843 | 0.201 | 0.130 |

[¥] Refer to figure 3 for this significant interaction effect.

Figure 3. Impacts of biomass incorporation on abundance of bacterial *amoA* at different soil depths in the short-term experiment.




** significant difference at $P \leq 0.01$

**Discussion**

The goal of this study was to assess the N retention potential and leaching dynamics of applied labelled ammonium fertilizer in soils with recent orchard biomass incorporation (short-term), and whether the legacy effects of this biomass incorporation alter N dynamics in the long-term. We found that *in-situ* biomass recycling prior to tree replanting significantly increased N fertilizer immobilization shortly after incorporation into the soil, especially within the topsoil (0-15 cm). In the longer term, we observed a 52% reduction in leaching potential of N fertilizer without increased immobilization of recently applied N fertilizer. Although there may be short term trade-offs associated with soil incorporation of woody biomass, the long-term environmental benefits are significant. Long term gains associated with this practice can have potential positive impacts on water quality, agricultural communities and growers trying to comply with increasing water quality regulations in California.

**Whole orchard recycling increases N immobilization without reducing fertilizer N leaching in the short-term**

It has previously been demonstrated that addition of organic amendments with high C:N ratios generally increase soil N immobilization, which may result in reduced availability of recently applied N fertilizers within recycled orchard soils (Culumber et al., 2018). Our findings confirm these results with higher gross and net N immobilization rates in an orchard recently amended with large amount of biomass. Net N mineralization rates have been shown to decrease with increasing immobilization rates associated with increased amounts of added C (Bengtsson et al., 2003; Binh and Shima, 2018) while ammonium utilization pathway gradually switch over time from net nitrification to immobilization as the C:N ratio of amendment increases (Feng and Zhu, 2017) . We did not detect differences in mineralization dynamics with biomass addition in the short-term experiment, indicating potential large immobilization within the microbial biomass with limited turnover soon after incorporation. This is corroborated by our results of increased immobilization within the microbial biomass which acted as a significant and immediate sink for recently added N as shown by elevated isotope signature from fertilizer in microbial biomass ($\delta^{15}N_{MB}$). Detection of greater values of $\delta^{15}N$ in the MBN pool with biomass addition might indicate greater competition for N in the recycled orchard soil in the short-term, with the high N demand required for the decomposition and assimilation of high C:N ratio (~160) orchard biomass.

As such, microbial utilization of orchard biomass, or any other low N content substrates, is often accompanied by the immobilization of inorganic N from the soil and heightened competition for N leading to increased microbial investment in N acquisition strategies (Avnimelech, 1999; Malik et al., 2020). This is particularly supported by the significantly uniquely higher MBN and $\delta^{15}N_{MB}$ values observed in the topsoil within the orchard biomass incorporation zone. This is likely attributed to an increase in microbial biomass carbon (MBC), associated with the substantial input of orchard biomass, and the stoichiometric necessity of assimilating additional N to balance the C:N ratio requirements of biomolecule synthesis. This is supported by a well-documented positive correlation between soil MBC and MBN across studies (Geisseler et al., 2010; Jahanzad et al., 2020). Thus, the increase in sustained soil microbial growth of the +biomass treatment and immobilization potential is likely associated with the additional energy and nutrients of incorporated orchard biomass within the topsoil (Bonanomi et al., 2011; Throckmorton et al., 2012).

Despite the significant short-term immobilization potential of N within the microbial biomass (Table 1), recently recycled orchards may still be prone to losses of applied N through leaching, as represented by similar $\delta^{15}$N nitrate values in the leachate of both +biomass and -biomass treatments. This may be due to a lack of adequate decomposition time for the newly incorporated orchard biomass as indicated by similar soil organic carbon (SOC) and soil organic matter (SOM) values across the soil profile, which may limit N sorption sites and soil water holding capacity, both important drivers of N retention. Higher activity and abundance of bacterial *amoA* in the lower soil layer (15-30 cm) compared to the topsoil could also be a driver of rapid N transformation into mobile nitrate, balancing out potential gain in N retention via immobilization at the soil surface. Other studies have linked high rates of nitrate leaching to the abundance and activity of bacterial *amoA* in saturated soils (Di et al., 2010; Di and Cameron, 2012; Galloway et al., 2003; Isobe et al., 2018). However, lack of significant difference in gross mineralization and significant increases in MBN with biomass addition indicate that this heightened *amoA* abundance might be more related to the increased short-term microbial assimilation of recently deposited $NH_4$-N fertilizer under woody biomass incorporation. Additions of orchard biomass, when combined with the application of $(NH_4)_2SO_4$ fertilizer, also resulted in a short-term decrease of nitrite reducing functional gene (*nirK*) abundance, perhaps indicating shifts in loss pathways towards leaching.

**Biomass recycling reduces nitrate leaching potential in the long-term**

While recently recycled orchards may still be prone to higher nitrate leaching potentials dues to recent soil disturbance for land preparation, we observed significantly lower nitrate leaching ten years after orchard biomass additions. Notably, there were no long-term significant difference in gross N immobilization (GNI) with biomass recycling and lower mineralization rates contributed to a significantly less negative net N immobilization (NRI) value in the +biomass treatment. Interestingly, soils exhibited marked increase in abundance of nitrite reductase *nirK* with biomass incorporation, which jointly may indicate a reduction in nitrate leaching potential because of increased gaseous loss of N (NO).

The decrease in nitrate leaching for recycled orchard soils may also be explained by higher water retention with SOC dependent improvements in soil physical and hydraulic properties. An increase of 30% in field water holding capacity has been reported for biomass amended soils at this site (Jahanzad et al., 2020). Biomass additions also increased the formation and stability of large macroaggregates (>2mm diameter) while also occluding a larger quantity of intra-aggregate SOC (Jahanzad et al., 2020). Previous studies have linked soil C amendments with improved water retention and lower nitrate leaching potentials, attributing the observed benefits to factors such as increased soil aggregate formation and stabilization (Colombani et al., 2020; Liu et al., 2017; Lu et al., 2020). Subsequently, improved soil structure increases the diversity of soil pore-size distribution as well as decreasing the prevalence of preferential hydraulic flow channels, which could in turn result in lower nitrate leaching potential.

Depending on C:N ratio of soil amendments and inherent soil characteristics, literature varies in terms of functional gene abundance and bacterial community responses. While several studies highlight a significant response of functional gene abundance to the soil amendments such as compost and biochar (Li et al., 2019; Lu et al., 2020; Wu et al., 2016), other studies report a neutral or negative correlation response between organic soil amendments and the presence of functional N cycling genes (Ouyang et al., 2018; Wessén et al., 2010; Yu et al., 2019). Several influential factors such as climatic conditions, soil type, soil physicochemical properties, and residue quality likely regulate responses of N cycling genes to soil amendments (Gonzalez Perez et al., 2014; Lin et al., 2018; Pereira e Silva et al., 2011). Due to the high C:N ratio of the orchard biomass incorporated, results are likely to shift across locations and recycling methodologies (i.e., particle size) in these irrigated landscapes. As such, further elucidating N pools variation and associated shifts in microbial functions with biomass addition across a co-management gradient and various woodchip sizes will be critical to tailor this practice to the various environments, increase benefits, and lower potential production tradeoffs.

## Conclusion

We present here the first exploration of shifts in N dynamics and leaching potential with whole orchard recycling practice in the California Central Valley on the short (one year) and longer term (ten years). Our results, coupled with previous reports of benefits for tree productivity and soil health (Jahanzad et al, 2020) highlight the potential of whole orchard recycling to harness soil ecosystems for sustainable perennial crop production systems and conservation of groundwater quality. Stacking practices such as implementing legume cover crops to add labile C and N before replanting and establishing zone-specific management strategies to prime mineralization in the tree row while catching leachable N in the alleyways would likely help offset short term tradeoffs. Proactive adaptation of N management practices such as early fertilization and rootzone placement of N fertilizer might also mitigate the potential need for higher fertilizer application rates than the standard recommendation for first year trees. Biomass recycling can therefore be considered as a promising long-term climate smart agriculture practice, which offers broad ecosystems services for adaptation to future climate variation while mitigating nitrate discharges to groundwater bodies during the orchard productive phase.

**Supplemental Material**

Supplementary Table S1. Presents soil chemical properties at the short-term and long-term experiments

Supplementary Table S1. Soil chemical properties at the short-term and long-term experiments

| Experiment | Short term | | | | Long term | | | |
|---|---|---|---|---|---|---|---|---|
| Depth | 0-15 cm | | 15-30 cm | | 0-15 cm | | 15-30 cm | |
| Treatment | -biomass | +biomass | -biomass | +biomass | -biomass | +biomass | -biomass | +biomass |
| TN (g kg$^{1}$) | 0.89 | 0.89 | 0.51 | 0.52 | 0.49 | 0.51 | 0.61 | 0.62 |
| SOC (g kg$^{1}$) | 9.68 | 10.40 | 5.00 | 5.10 | 6.21 | 8.53$^{**}$ | 7.86 | 11.50$^{**}$ |
| SOM (g kg$^{1}$) | 18.68 | 19.34 | 12.42 | 13.43 | 11.00 | 15.4$^{**}$ | 15.30 | 18.64$^{*}$ |
| pH | 6.18 | 6.69$^{*}$ | 6.64 | 6.76 | 6.96 | 6.89 | 6.82 | 6.75 |
| EC (dS m$^{-1}$) | 0.19 | 0.21 | 0.2 | 0.18 | 0.54 | 0.54 | 0.48 | 0.51 |
| K (mg kg$^{1}$) | 224 | 238 | 168 | 164 | 450 | 458 | 485 | 507 |
| Ca (mg kg$^{1}$) | 1106 | 1250$^{*}$ | 948 | 1005 | 60.49 | 61.08 | 62.11 | 68.54 |
| Mg (mg kg$^{1}$) | 234 | 231 | 16.9 | 17.9 | 17.73 | 18.04 | 23.09 | 23.56 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Na (mg kg$^{-1}$) | 20.9 | 21.5 | 23.1 | 22.4 | 18.04 | 21.32* | 19.21 | 20.95 |
| CEC (meq$^{\dagger}$) | 9.46 | 10.16 | 7.01 | 7.34 | 8.16 | 8.45 | 9.25 | 10.14 |

EC, electrical conductivity; CEC, cation exchange capacity († Per 100 g of soil); TN, total nitrogen; SOC, soil organic carbon; SOM, soil organic matter; * significant difference at $P \leq 0.05$; ** significant difference at $P \leq 0.01$

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