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Performance and Plant-Available Nitrogen (PAN) Contribution of Cover Crops in High Elevations in Hawai‘i

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Introduction

Cover crops are the backbone of sustainable cropping systems: they can prevent soil erosion, reduce nutrient leaching, add organic matter, improve soil health, suppress weeds, and reduce insect pests and diseases (Bowman et al. 2007). In addition to these well-established benefits, leguminous cover crops can contribute significant amounts of nitrogen to crop production. However, farmers need better information to accurately estimate the nitrogen contribution from legumes so they know by precisely how much to reduce fertilizer rates. Selecting a cover crop species appropriate for a specific growing season and conditions is important to achieve maximum benefits from cover cropping. Previous studies have demonstrated benefits of cover crops for sustainable crop production in lower elevations in Hawai‘i (Radovich et al. 2009, Radovich 2010, Wang 2012, Wang and Marahatta 2009). The objectives of this project are to evaluate 1) growth performance, 2) plant-available nitrogen (PAN) contribution, and 3) soil health enhancement of different species of cover crops at higher elevations in Hawai‘i.

Cover crop field day, Lālāmilo Research Station

Nematodes as soil health indicators

Nematodes have proven to be good soil health bio-indicators (Ferris 2010, Neher 2001, Wang and McSorley 2005). Although bacteria and fungi are the primary decomposers, when organic matter, including cover crop residues, is incorporated into the soil, these microbes immobilize inorganic nutrients in the soil, making nutrients unavailable for plant uptake. As an extension of these decomposition channels, when bacterivorous and fungivorous nematodes graze on these microbes, they give off CO_2 and NH_4^+ and other nitrogenous compounds, affecting C and N mineralization directly (Ingham et al. 1983). Nematode excretion may contribute up to 19% of soluble N in soil (Neher 2001). Therefore, a healthy soil food web should sustain nematodes with different life strategies and feeding behaviors ranging from fast-growing and -breeding bacteria-feeding nematodes at the bottom of the food chain to slow-growing, slow-reproducing predaceous nematodes at the top for efficient mineralization of nutrients accumulated in organic matter and microbes. Besides contributing to

N mineralization, the abundance of many free-living nematodes, especially bacterivorous, omnivorous, and predatory nematodes, also was found to correlate with concentrations of many other soil nutrients, such as P, K, Ca, Mg, Fe, Cu, Mn, Zn, and Na, as well as soil organic matter and cation exchange capacity (Wang et al. 2004), suggesting the possibility that nematodes mineralize many other soil nutrients. For more explanation of why nematodes are good indicators for soil health, please refer to Wang (2010).

Materials and Methods

A field trial was conducted at Lālāmilo Experiment Station, Waimea, Hawai‘i, between November 2013 and February 2014. Winter cover crops hairy vetch (*Vicia villosa*), Austrian winter peas (*Pisum sativum* var. *arvense*), bell beans (*Vicia faba*), annual ryegrass (*Lolium multiflorum*), Cayuse oats (*Avena sativa*), a mixture of annual ryegrass and hairy vetch, and a commercial Soil Builder Mix composed of bell bean, BioMaster peas and Arvika peas (*Pisum sativum*), purple vetch (*Vicia Americana*), hairy vetch, common vetch (*Vicia sativa*), and Cayuse oat were seeded in November 2013 in individual plot 20 × 20 ft^2 in size. Treatment plots were arranged in a completely randomized design, with 4 replications for all cover crops tested except for oat and Soil Builder Mix, which were planted in one plot each. Seeding rates for hairy vetch, Austrian winter peas, bell beans, annual ryegrass, oats, and Soil Builder mix were 40, 100, 200, 100, 90, and 100 lb/acre, respectively. A mixture of 70 lb/acre annual ryegrass and 30 lb/acre vetch was used for the ryegrass-and-vetch combination. Live culture of nitrogen-fixing *Rhizobacteria*, typically *Rhizobium leguminosarum* bv *viceae*, was used to inoculate the bell beans, Austrian winter peas, vetch, and ryegrass-and-vetch mixture prior to sowing. All cover crop seeds were purchased from Peaceful Valley (http://www.groworganic.com/). These cover crop seeds are also available at Koolau Seed (Phone: 808-239-1280), Johnny's Seeds (http://johhnyseeds.com/), LA Hearne (http://hearneseed.com/), and Snow Seed (https://www.snowseedco.com/).

Cover crop biomass

Data on percent ground cover of cover crop, weeds, and bare ground in each plot were recorded 2 months after seed sowing. Above-ground cover crop biomass was weighed at 70 days after seed sowing by cutting

Cool-season cover crop establishment at 2 months after planting in at Lālāmilo Experiment Station during winter season of 2013–2014. A) hairy vetch, B) Austrian winter pea, C) bell beans, D) annual rye grass (competed by weeds), E) oat, F) mixed planting of hairy vetch and annual ryegrass, F) Soil Builder Mix.

plant shoots at ground level from three sets of 50 × 50 cm^2 quadrats randomly placed in each plot. Whole-plant samples collected from each plot were mixed and subsampled to determine total nitrogen (N) and carbon (C). The subsamples of fresh cover crop biomass were sliced into approximately 15-cm-long pieces and frozen in 3.8L (1 gal) Ziploc® freezer bags prior to incubation for mineralization. Incubation here represents the process of storing mixtures of soil and cover crop residue at room temperature with enough moisture to allow breakdown of cover crop tissues and release plant-available nutrients into the soil. Frozen cover crop biomass was then sliced into smaller pieces (10 to 15 mm in length) with a serrated knife and minced repeatedly prior to incubation. Another set of cover crop subsamples was dried at 70°C to determine the dry matter content.

Plant-available N (PAN) analysis

Soil used for the mineralization test (pH approximately 6.5) was collected from each cover crop plot corresponding to the cover crops tested. This was silt loam soil (Series: Waimea, Order: Andisol, Family: Medial, Amorphic, Isothermic Humic Haplustands) derived from volcanic ejecta such as ash, pumice, and cinder (Hue et al. 2007, Deenik and McClellan 2007). When necessary, additional moisture was added to achieve approximately 25% soil moisture by spreading out the soil and moistening through repeated misting and mixing. The defrosted, sliced cover crop biomass samples were mixed with 660g moist soil (equivalent to approx. 500g dry soil), then placed in 0.9L (1-quart) Ziploc® freezer bags. Two small holes were punched in each bag, into which drinking straws were inserted to allow air entry into bags during the incubation. Cover crop addition rate was 10g dry matter per kg soil (1.0%). Large amounts of soil used for incubation allowed sequential sampling of soil without changing soil volume significantly. The large incubation bags also helped to maintain moisture for extended incubation periods.

Plant-available N mineralized from cover crop residues was determined using two steps. Soil nitrate-N accumulation after 28 and 70 days of incubation at 22°C was measured in subsamples collected from the incubation bags. Plant-available N released from cover crop residues was corrected based on nitrate-N recovered in the soil-only control bags, and expressed as a percentage of cover crop total N input (Sullivan et al. 2011).

$$\textit{PAN (\% of cover crop total N)} = [(\textit{soil } NO_3\textit{-N with cover crop} - \textit{soil only } NO_3\textit{-N}) \div (\textit{cover crop total N added to the soil})] \times 100$$

The following prediction equation was originally developed by Vigil and Kissel (1991) to predict PAN release from crop residues in Kansas:

$$\textit{PAN (\% of cover crop total N)} = -53.44 + 16.98\sqrt{(\textit{cover crop tissue \% N} \times 10)}$$

The accuracy and precision of Vigil and Kissel's equation (VK Eqn) in Waimea soil was evaluated. Since the VK equation suggests that PAN release by cover crop is a function of tissue N content, regression analysis of tissue N concentration vs. PAN% measured was conducted to evaluate the validity of VK equation in Waimea soil.

Soil health analysis using nematodes as bio-indicators

Composite of 4 soil sampling cores collected from each field plot of hairy vetch, Austrian winter peas, bell beans, annual ryegrass, annual rye grass and vetch mixture, and soil builder mix were collected prior to

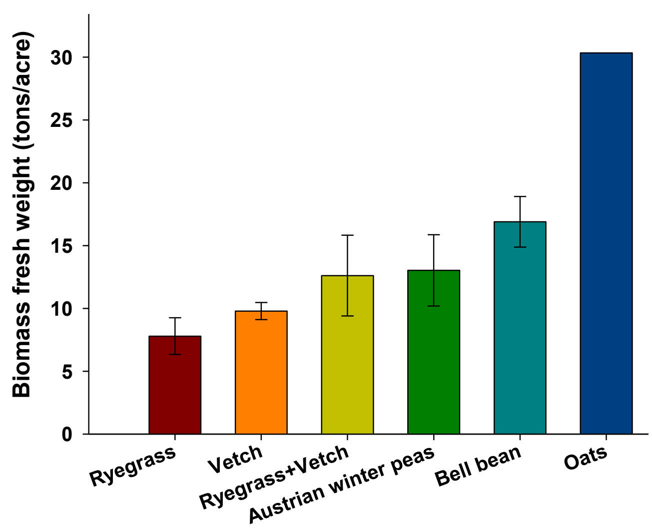

Fig 1. Biomass generated by the cover crops listed. Means are average of 4 replications. Bar above each column is standard error bar.

cover crop planting (first week of November 2013), 2.5 months after cover crop planting (February 5, 2014) and about 1 month after cover crop incorporation (April 1, 2014) for nematode analysis. Nematodes were extracted from a 250-cm^3 subsample of soil per sample by elutriation and centrifugal flotation method. All extracted nematodes were identified to the genus level wherever possible, counted, and assigned to one of the six trophic groups: algivores, bacterivores, fungivores, plant-parasitic nematodes, omnivores, or predators (Yeates et al. 1993).

Statistical analysis

Analysis of variance (ANOVA) of biomass, ground cover, tissue nutrients, PAN%, and actual PAN contribution by different cover crop species was performed using PROC GLM in SAS 9.1 statistical software (SAS Institute Inc. 2003). Means were separated using Tukey's HSD. Simple regression was conducted to evaluate the relationships between tissue N content and PAN% from different species of cover crops included in the trial. Statistical significance was obtained at 95% confidence level ($\alpha = 0.05$).

Nematode abundance data for each trophic group were log-transformed ($\log_{10}[x + 1]$) before analysis of variance (ANOVA). Untransformed means were presented, and separated using the Waller–Duncan k ratio (k = 100) t-test ($P \leq 0.05$).

Results and Discussion

Cover Crop Biomass and Ground Coverage

Among the cover crop species examined, Cayuse oat produced the highest biomass, followed by bell bean and Austrian winter peas (Fig. 1). Biomass production in annual ryegrass + vetch was similar to bell bean and Austrian winter pea, but higher than either vetch or annual ryegrass planted alone. Percentage of ground cover of annual ryegrass alone was the poorest among all species examined and resulted in highest weed coverage ($P < 0.05$; Fig. 2A).

Performance of oats was superior among all the species tested, with about 30 tons/acre fresh weight and 95% ground coverage (Figs. 1 & 2). Except for the annual ryegrass, the biomass production of all the cover crop species tested in this study was comparable to the biomass production of these species reported in the cover crop database of University of California (SAREP UC Davis). This indicated that most of these cool-season cover crops tested can be adapted to high-elevation conditions, such as Waimea in Hawai‘i during the winter-time. However, biomass production of annual ryegrass was 4-8 times lower than that reported in the cover crop database of University of California.

C, N, and Plant-Available Nitrogen (PAN)

Total carbon content in all cover crops tested was similar except for annual ryegrass (Fig. 2 B). However, % total N content in oat was lowest compared to other

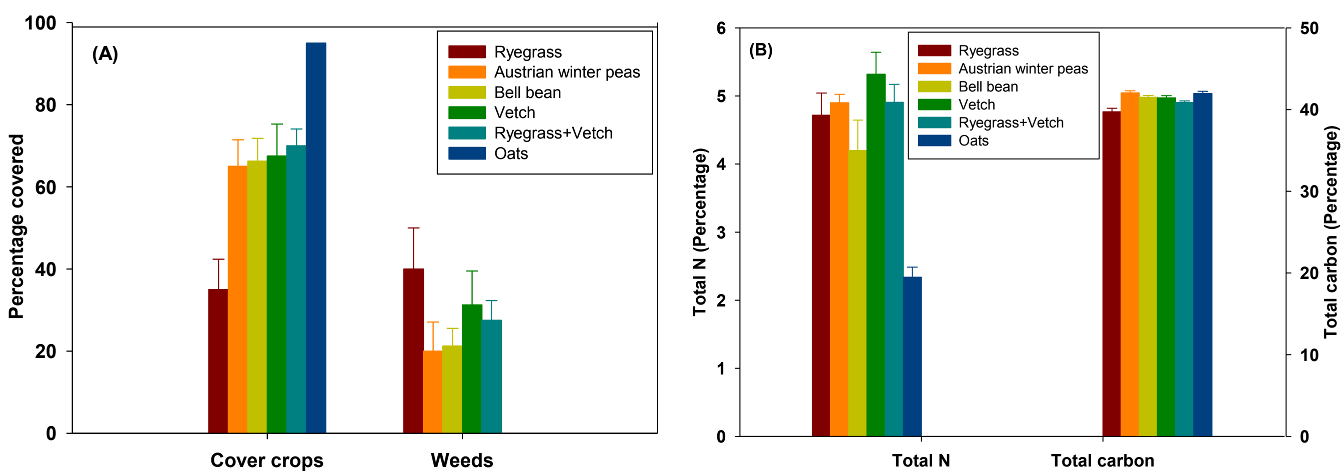

Fig 2. A) Cover crops and weed coverage in different cover crop plots, B) tissue nitrogen and carbon content of different species of cover crops.

species tested (Fig. 2 B). Nitrate (NO_3-N) release by all the species tested in this trial except for oat was comparable at 4- or 10-week incubation periods. Oat released only about one third of NO_3-N of the other cover crops during the same incubation period. Of the total NO_3-N released during the 10-week incubation period, most of the NO_3-N (85–90%) was released in the first 4 weeks across the species (Fig. 3A). A similar trend was observed for the percentage of PAN released from different species of cover crops over 70 days (Fig. 3B).

The regression analysis showed that the calculated percentage of PAN released by the residue of different cover crop species is positively correlated (*R^2 = 0.54, $P<0.001$* at 28 days and *R^2 = 0.502, $P<0.001$* at 70 days) to tissue N content (Fig. 4A). The calculated % of PAN is positively correlated also with the VK equation-predicted PAN (*R^2 = 0.54, $P<0.001$*) (Fig. 4B). This suggests that the VK equation can be used to calculate the % of PAN released by cover crop residue. However, further study is required to obtain an equation to better estimate plant-

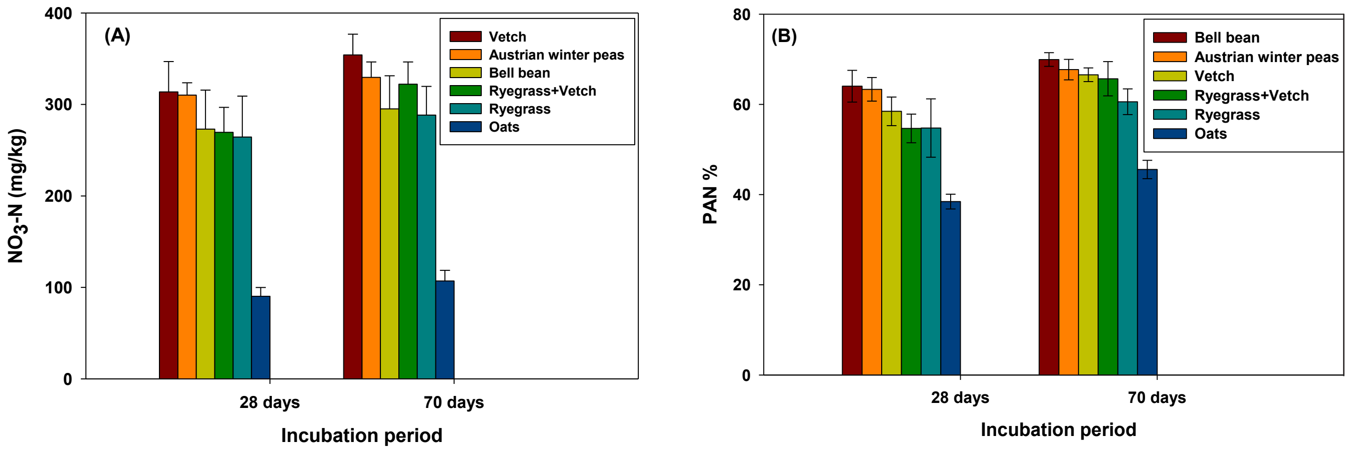

Fig. 3. A) NO_3-N released and B) % of PAN released by different species of cover crops over time.

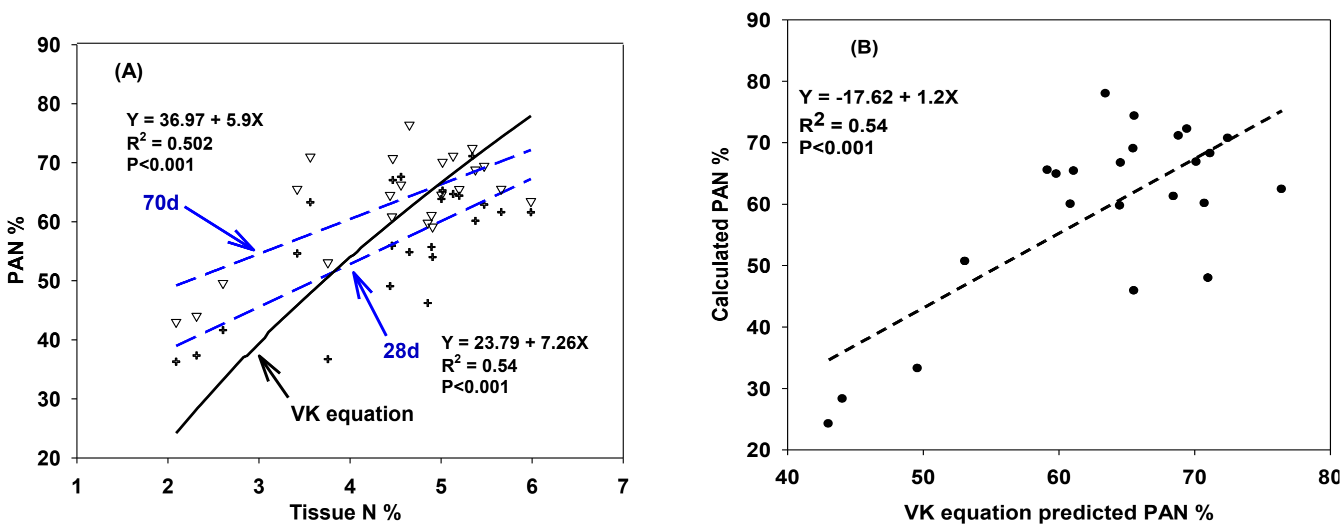

Fig. 4. (A) Correlation between cover crop residue total N concentration and plant-available N (% PAN) determined in laboratory incubations in moist soil, (B) Correlation between calculated plant-available N (% PAN) and VK equation-predicted PAN %.

Table 1. Actual plant-available nitrogen (PAN) contributed by cover crop residues incubated in Waimea soil for 28 or 70 days.

	Actual PAN (lb/acre)	
Species	**28 days**	**70 days**
Austrian winter pea	106.24 ± 14.26 a	113.56 ± 17.72 a
Cayuse oat	94.23 ± 10.02 ab	111.71 ± 12.11 ab
Bell bean	95.45 ± 16.10 ab	103.68 ± 14.74 abc
Vetch	68.72 ± 9.36 ab	77.23 ± 7.02 abc
Annual ryegrass	71.38 ± 6.16 ab	74.18 ± 5.82 bc
Annual ryegrass+vetch	56.53 ± 6.68 b	70.16 ± 4.94 c

available nitrogen more precisely by integrating other factors besides % N.

The important information for growers to know about specific cover crops is the actual PAN contributed. Actual PAN is attributed to the biomass production, tissue N content, and percentage of PAN released during the incubation period. The actual PAN (lb/acre) contributed by individual cover species incubated in soil collected from at Lālāmilo over the 28-d and 70-d periods are shown in Table 1.

The actual amount of PAN provided by cover crop residues after 10 weeks is important information, as this is the common growing period of short-term vegetable crops where most N is needed. After 10 weeks of incubation, Austrian winter peas and Cayuse oats contributed the most PAN lb/acre, whereas ryegrass and the mixture of ryegrass and vetch contributed the least PAN among the cover crops tested. Therefore, despite the fact that Cayuse oat contained the lowest % N in its tissues, it contributed a similar amount of N available for plant uptake as Austrian winter pea. On the other hand, even though a mixture of annual ryegrass and oat contributed a similar amount of plant biomass as Austrian winter pea, it contributed less actual PAN than Austrian winter pea and Cayuse oat. This implies that cover crop biomass, % tissue N content, and % PAN released by a cover crop are important for the prediction of actual plant-available nitrogen for the succeeding crop.

Nematodes as Soil Health Indicators

In general, all cover crops tested tended to support more bacteria-feeding nematodes as compared to the bare ground control at 2.5 months after planting (Fig. 5A). The cover crops were not significantly different in this regard; however, hairy vetch supported the highest numbers of bacterivores, indicating higher activities of bacteria decomposition, whereas AR, AWP, and BB provided relatively balanced fungal and bacterial decomposition channels, as indicated by similar abundance of these nematodes.

The performance of Soil Builder Mix in enhancing free-living nematodes was average in this experiment and resulted in the lowest number of genera of nematodes detected (nematode richness) (data not shown). This data did not support the theory that higher plant diversity above ground would support higher diversity below ground. It is possible that this mixture of cover crop species is not the most suitable for Waimea soil or climate conditions. We did not measure the N content and biomass produced by SB, as the treatment was not replicated. At one month after soil incorporation of the cover crops, a general increase in the abundance of bacterivorous and fungivorous nematodes (Fig. 5B) as compared to those during the cover crop growing period (Fig. 5A) was observed. This is an indication of an increase in microbial activity after organic matter incorporation into the soil from cover crops or weeds. A similar trend was observed where the bare ground control supported the lowest number of free-living nematodes, but no significant difference was detected. At this sampling date, Soil Builder supported the lowest number of omnivorous nematodes, while AR+V had the highest count of omnivorous nematodes. Longer-term cover cropping history is needed to further evaluate the impact of cover cropping on soil health. Nonetheless, it is promising to see, regardless of cover crop species, that cover cropping supported more soil microbial activity as compared to the bare ground control, with minimal weed establishment.

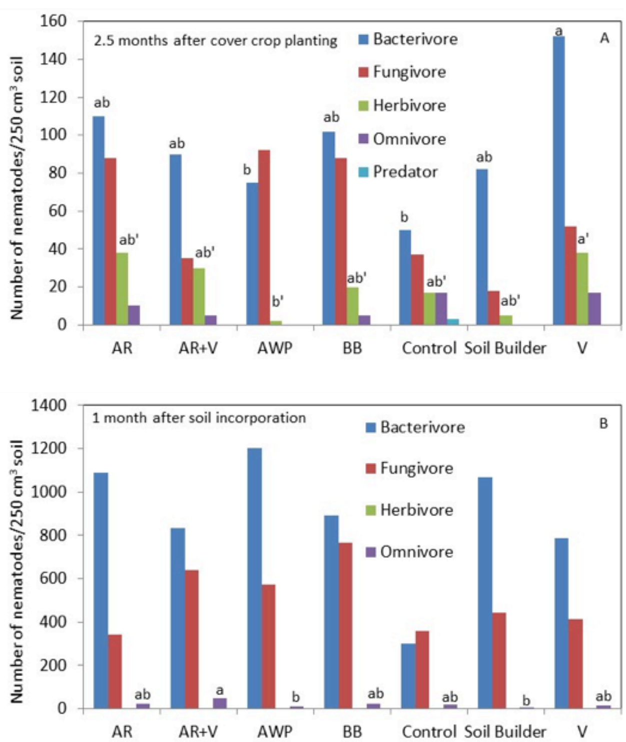

Fig. 5. Abundance of nematodes in the trophic groups of bacterivores, fungivores, herbivores, and omnivores at A) 2.5 months after cover crop planting, and B) one month after soil incorporation of 6 cover crop treatments and a bare ground (control) at Lālāmilo Experiment Station. The 6 cover crops are annual ryegrass (AR), Austrian winter pea (AWP), bell bean (BB), hairy vetch (V), and Soil Builder (SB) cover crop mix. Means are an average of 4 replications, except in the case of SB Mix, which had only one replication. Means for each trophic group followed by the same letter(s) are not different according to the Waller-Duncan k-ration (k=100) t-test.

Summary

Biomass production and ground coverage of the cool-season cover crops tested suggest that Cayuse oat is a suitable cereal cover crop, while bell bean and Austrian winter pea are suitable leguminous cover crops for winter in Waimea or other locations with similar climatic and soil conditions. The results also suggest that annual ryegrass is not an ideal cereal cover crop for this region. Also, combining oat with vetch may potentially be a better cover crop mixture in terms of its benefits. The results of % PAN released by different species of cover crops suggest that leguminous cover crops contribute a higher percentage of PAN compared to oats. However, actual PAN contribution from oats was comparable to leguminous species, as it has greater biomass. Most of the PAN from all the species tested was released within in the first 4 weeks of soil incubation; hence the N-management strategy subsequent to cover cropping should be implemented to maximize the use of PAN released by cover crops. Additional N requirements for crop plants can be supplemented later in the season, so as to minimize nitrate leaching. The balanced abundance of bacterivorous and fungivorous nematodes in bell bean and Austrian winter pea and high abundance of bacterivorous nematodes in vetch might partially explain the relatively high actual PAN released from these cover crops.

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