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Soil Microbial Enzyme Responses to Changes in Temperature and Nutrient Additions across Hawaiian Gradients in Mineralogy and Nutrient Availability

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by

Taylor Leigh McCleery

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ABSTRACT OF THE THESIS

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University of California, Los Angeles, 2014

Professor Daniela Cusack, Chair

Microbial enzyme activities are the direct agents of organic matter decomposition, and thus play a crucial role in global C (C) cycling. Global change factors like anthropogenic nutrient inputs and warming have the potential to alter the activities of these enzymes, with background site conditions likely driving responses. We hypothesized that enzyme activities in sites with high background soil nutrient and/or C availability would be less sensitive to nutrient additions than nutrient-poor sites. We also hypothesized that sites poor in nutrients and/or C would show greater sensitivity to changes in temperature. To test our hypotheses we used long- and short-term nutrient additions combined with laboratory temperature incubations to assess changes in enzyme activities for 8 common soil enzymes that acquire nitrogen (N), phosphorus (P) and C from organic matter. We collected mineral soils (0-10 cm depth) from 8 Hawaiian sites that provided maximum variation in nutrient availability and background soil C. Soils were sieved, pooled by site, and homogenized prior to a laboratory addition of a simple C (sucrose), N, and/or P in full factorial design. The 8 soils also were incubated at 7 temperatures from 4 – 40 ºC.

We found that the laboratory fertilizations altered enzyme activities, and that temperature sensitivities varied significantly among sites. Across the 8 sites, laboratory sucrose+N and sucrose+NP additions increased C-, N-, and P- acquiring enzymes activities ($p < 0.05$), with the strongest effect, as predicted in nutrient and C-poor soil. Phosphorus-acquiring enzymes were the most sensitive to these additions, while C-acquiring enzyme activities were less responsive. Results suggest that C-, N-, and P-acquisition enzyme activities respond positively to added nutrients across sites, regardless of background nutrient status. In particular, P-acquisition was broadly sensitive to N addition even in relatively N-rich soils, whereas C- and N-acquisition activity appeared to be generally sensitive to N and P. Overall, enzyme activities responded most strongly to the addition of sucrose+N or sucrose+NP. Temperature sensitivity varied significantly across sites and among enzymes, with greater temperature sensitivities for enzymes that acquire N and P in wetter sites than drier sites. In contrast to our hypothesis, enzyme temperature sensitivities were strongest for soils from relatively nutrient- and C-rich forests, than for drier sites poorer in soil nutrients and C. Enzyme Q_{10} were measured using the Arrhenius equation to determine temperature dependency of reaction rates. Enzyme Q_{10} values followed a log linear relationship and were most sensitive to changes from the 10 °C to 20 °C range as opposed to warmer temperatures ($p < 0.05$). Enzyme responses to changes in climate such as nutrient additions and warming will likely have negative implications for soil C storage as enzymes generally responded positively to warming and nutrient additions.

The Thesis of Taylor Leigh McCleery is approved

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Tropical Soil Enzyme Activity and Global Change

Soil Enzymes and Global Change

i. What are Soil Enzymes?

Soil microbial enzymes play an important role in the decomposition and formation of soil organic matter, central processes in the global carbon (C) and nutrient cycles (Sinsabaugh et al. 2008). Plant litter and microbial debris is composed of C rich bio-macromolecules such as cellulose, hemicellulose, pectin, chitin, lignin, and tannin (Hedges and Oades 1997, Gessner et al. 2010). This debris is both structurally complex and highly diverse, necessitating the combined activity of many microorganisms to decompose it. Microorganisms and plants release enzymes into the soil to decompose these C rich tissues and reduce them to soluble substrates (e.g. sugars, amino acids, NH_4^+, PO_4^{-3}) (Burns 1978, Burns and Dick 2002). By decomposing these complex plant materials, enzymes return nutrients in mineral form into the soil for plant and microbial uptake (Sinsabaugh 1994, Schimel and Bennett 2004, Liang and Balser 2012). Mineralization of labile compounds by soil enzymes can therefore mediate plant production, as well as drive global C and nutrient cycles, including atmospheric gas composition, through the degradation of organic matter compounds (Burns et al. 2013).

Soil microorganisms utilize soil enzyme strategies that minimize C and nutrient loss via respiration and waste, while maximizing production of biomass (Allison et al. 2011). However, there is a cost and benefit of enzyme production for microorganisms. The key costs of enzyme production are the metabolic energy required for protein synthesis and excretion and the C and nutrient content of the enzymes themselves (Friedel and Scheller 2002). The benefit is the dual function of degrading organic material into simpler forms and acquiring resources for the

enzyme producers (Burns 1982). Extracellular enzymes can target nearly every macromolecule on earth, including proteins (proteases), carbohydrates (amylases, cellulases), amino sugar polymers (chitinases), organic phosphates (phosphatases), and lignins (oxidases, peroxidases) and degrade these compounds into simpler compounds (Burns 1978, Allison et al. 2007). The primary use of enzymes from the viewpoint of the producer organism is the acquisition of limiting nutrients, such as N and P, via enzyme foraging strategies such as direct uptake of simple resources or nitrogen fixation (Allison et al. 2011) to provide necessary nutrients for growth. Without extracellular enzymes, C and nutrient cycles and the organisms dependent on their activity would therefore come to a standstill as complex C compounds would not be decomposed. These soil enzyme processes are reliant on environmental conditions such as soil C chemistry and nutrient status that affect the costs and benefits of enzyme production and activity.

There are two general groups into which soil enzymes can be divided: (1) hydrolytic enzymes which control the acquisition of C, N, and P for primary metabolism; and (2) oxidative enzymes responsible for the degradation of poor-quality, chemically complex compounds like lignin, which do not directly support growth, in cometabolic acquisition of non-C nutrients (Sinsabaugh and Moorhead 1994, Sylvia et al. 2004). Primary metabolism refers to the use of a substrate as an electron donor for C and energy, which are used for microbial growth and result in increased decomposition. Cometabolic processes do not provide C and energy directly for growth, but rather have the secondary effect of releasing essential nutrients from structural tissues (Boopathy 2000). Hydrolytic enzyme activities are substrate or bond-types specific, and can degrade a variety of complex compounds (Nannipieri et al. 2002), while oxidative enzymes are not substrate specific, hence extremely powerful. However as oxidative enzymes are specialized for the decomposition of such a complex compound such as lignin, they are not

always the most efficient decomposers of all compounds (Kirk and Farrell 1987). Soil enzymes and the processes that control and produce them are essential for the breakdown of compounds necessary for the regulation of nutrient cycles, such as the C cycle. These processes contribute to soil C cycling in the tropics, which make up a large portion of global C storage.

ii. Tropical Soil Enzyme Activity

Tropical soils play an important role in global C cycling, making understanding of processes that regulate soil C cycling in tropical ecosystems broadly significant. Because of high plant productivity in tropical forests, which accounts for a large portion of global terrestrial C storage and cycling, tropical forests are thought to play a large role in anthropomorphizing the atmosphere against rising CO_2 (Malhi and Phillips 2004, Chave et al. 2008, Lewis et al. 2009, Gerber et al. 2010). In particular, tropical forests have higher potential C uptake rates than forests in temperate and boreal biomes (Brown 1995, Watson et al. 2000), with 40% of terrestrial biomass C in tropical forests (Dixon et al. 1994). Because net primary productivity is high in the tropics, litterfall represents a large flux of C and nutrients into the soil. Variations in litterfall may alter the activity of soil enzymes. For example, a litter decomposition study by Allison and Vitousek (2004) found that variations of litter type within the tropics corresponded with differing soil enzyme activities, indicating that differences of litter types are a significant predictor of enzyme responses. This is because in general, global enzyme activity is sensitive to changes in the C:N:P acquisition ratio (Cleveland and Liptzin 2007). Specifically, enzymes in tropical forests which are typically rich in N, direct more energy to acquiring and cycling P in highly weathered soils (Sinsabaugh et al. 2008). Additionally, microbial processes and production of enzymes are sensitive to N:C ratios with high N:C requirements and low N:C availablity in plant litter and SOM (Sylvia et al. 2004). A study in an urban-remote forest gradient in Puerto Rico

found that soil N levels were the strongest predictors of soil enzyme activities (Cusack 2013). These studies indicate that enzyme activities are sensitive to nutrient ratios present in soils and leaf litter, which has potential implications for global carbon storage.

High temperatures and relative consistency in warm temperatures create optimal conditions for tropical enzymes (Vitousek 1984, Schlesinger and Andrews 2000). These characteristics combine to create rapid decomposition rates in tropical forests (Parton et al. 2007, Adair et al. 2008). One study in tropical forests in Puerto Rico found that there was greater hydrolytic enzyme activity and a subsequent decrease in labile C compounds in SOM in a lower-elevation forest, while oxidative activity increased in the higher-elevation forest which corresponded with a decrease in aliphatic C (Cusack et al. 2010). Another study by Cusack found that sites with high precipitation and high lignin content had slower long-term decomposition and slower decomposition of high lignin litters (Cusack et al. 2009). Rates of decomposition in tropical forests are therefore likely sensitive to abiotic and biotic factors, such as precipitation, elevation, and chemical complexity of litter.

Tropical enzyme activity likely differs from the responses of temperate and arid ecosystems. Studies by Rabinovich et al. (2004) and Baldrian (2006) found that oxidative enzymes are generally produced by a variety of basidiomycetes. These organisms are most commonly found in mid- to high-latitude forests where the composition of leaf litter is high in lignin and the soil is acidic. Within these mid- to high-latitude ecosystems, phenol oxidase and peroxidase activities generally increase with secondary succession. Arid alkaline soils also have optimal pH condition for phenol oxidase and peroxidase activities (Stursova and Sinsabaugh 2008). A study in high-latitude peat lands found that phenol oxidase activity controlled organic matter mineralization and therefore CO_2 fluxes (Freeman et al. 2001). Overall, soil organic

matter content is greatest in high-latitude ecosystems where phenol oxidase and peroxidase activities are limited by low pH, low temperature and low oxygen availability (Sinsabaugh et al. 2008). In contrast, arid ecosystems have the lowest SOM content due to low rates of primary production, and high pH which is optimal for phenol oxidase and peroxidase activities (Collins et al. 2008). Soil enzymes in temperate and arid ecosystems are likely limited by differences in pH and lignin content.

Soil Enzyme Interactions with Soil C

The large storage of C in terrestrial ecosystems indicates potential large losses or uptake depending on ecosystem responses to global climate change factors. Specifically, terrestrial ecosystems store about 2100 Pg C in living organisms, litter and soil organic matter (Ruesch and Gibbs 2008). Soils themselves contain 2300 Pg of C in the top three meters alone (Jobbagy and Jackson 2000). Tropical areas have the potential to store a large amount of global C. In particular, tropical areas represent more than half of the global C uptake (Pan et al. 2011). Tropical soils alone store 471 Pg of C (Pan et al. 2011), with 30-60% of the ecosystem C stored in soils (Don et al. 2011). The relatively high temperatures and abundant rainfall allow optimal conditions for C uptake through photosynthesis (Luyssaert et al. 2007, Beer et al. 2010), but also rapid release through respiration and decomposition (Luyssaert et al. 2007). Because annual fluxes into and out of tropical ecosystems are so large compared to other ecosystems, a change in the balance of C due to fluctuation of temperature, rainfall, or nutrients can have significant impacts on soil C storage and corresponding atmospheric CO_2 levels.

The regulation of soil carbon storage is dependent upon the chemical composition of soil organic matter and the processes that decompose it. Lignin and cellulose are the biggest

contributors to living terrestrial biomass and are therefore important C suppliers to soil organic matter as the plant litter is decomposed (Crawford 1981). Because litterfall represents a large input of C and nutrients, it has been a major area of research (Aber et al. 1990, Sinsabaugh et al. 1993, Hobbie 1996, Berg 2000). The decomposition of this plant material has a strong influence over the carbon balance (Rustad 1994, Schlesinger and Lichter 2001). The release of nutrients from decaying plant litter may increase soil fertility, whereas plant litter that is not decomposed immobilizes available nutrients and reduces rates of carbon and nutrient cycling and plant growth (McGrath et al. 2000, Kwabiah et al. 2001). The balance between rates of decomposition and net primary production are the main controls on soil C stocks and influences the potential for ecosystems to sequester anthropogenic CO_2 from the atmosphere (Potter and Klooster 1997, Richter et al. 1999, Freeman et al. 2001, Schlesinger and Lichter 2001). Decomposition processes that regulate the storage of carbon in soils versus loss to the atmosphere can help us understand how soil carbon storage might change with future changes in our climate.

Overview of Global Temperature and Nutrient Change in the Tropics

Interacting global change factors such as warming and changes to nutrient cycles have the potential to alter tropical enzyme activities, microbial communities, and functioning. Changes to ecosystem functioning may be due to increasing readily available nitrogen caused by anthropogenic activities. Although nitrogen is very abundant in the atmosphere, most is not in the form of reactive nitrogen that is readily available for organism uptake (Bernhard 2012). Nitrogen is a fundamental nutrient for plant and organism growth and is therefore in constant demand. However, nitrogen is in short supply in forms that can be easily taken up by plants (Gruber and Galloway 2008). Alternately, the nitrogen applied as fertilizer and used for food production is biologically, photochemically, and radiatively active N compounds and is emitted

to the atmosphere during fossil-fuel combustion. These processes are supplying more nitrogen to the system than natural biological fixation on land (Gruber and Galloway 2008). Due to the advancement of crop production as a result of nitrogen fertilization, since 1970, human population has increased by 78% and reactive nitrogen levels have increased by 120% (Galloway et al. 2008). Because of rising food and energy demands, from 1860 to 1995 reactive nitrogen increased from ~16 Tg N in 1860 to 156 Tg N in 1995. This change increased even further from 156 Tg N yr^{-1} in 1995 to 187 Tg N yr^{-1} in 2005 (Galloway et al. 2008). These increases in reactive nitrogen are likely to have serious implications on global and tropical ecosystem structure and functioning.

Until recently, N deposition was considered a potential problem only for temperate forests, however now the highest rates of increase are likely to occur in tropical rainforests (Galloway et al. 2004). Temperate forests have naturally low N availability that limits productivity, so the addition of reactive nitrogen is likely to have large effects (Aber et al. 1989, Aber 1992, Hietz et al. 2011). Conversely, N is often not a limiting nutrient in highly weathered tropical forest soils (Walker and Syers 1976, Martinelli et al. 1999), so enhanced N deposition is adding nitrogen to forests initially rich in N. Observations in tropical forests over the last half century indicate that tropical nitrogen accumulates and recycles large quantities of N relative to temperate forests, shown by plant and soil nitrogen to phosphorus ratios and the export of bioavailable N at the ecosystem scale (Hedin et al. 2009). Even in the tropics, the increase in biologically available N has the ability to alter productivity, C storage, nutrient cycling, and species compositions (Vitousek 1997).

Changes in N availability in the tropics are likely to have adverse effects on ecosystem cycling, specifically C storage. Despite a lack of increased NPP with enhanced N availability, a

number of recent studies show that increased N availability in humid tropical forests has the ability to increase C storage (Luo et al. 2006, Ouyang et al. 2008, Cusack et al. 2011). One study by Cusack et al. (2011) found that belowground C cycling was sensitive to nitrogen additions, as there were increases in mineral soil C and declines in labile soil C in tropical forests of Puerto Rico. In fact, N addition can lead to an altered distribution of soil organic C between labile and chemically protected pools and increased mineral-associations (Ouyang et al. 2008, Cusack et al. 2011). Changes to the charge of soil organic matter may alter the adsorption and desorption of C minerals, leading to long-term storage (Sollins and Radulovich 1988, Chorover and Sposito 1995). Increases in soil C storage may also be due to a decrease in soil respiration that may occur with N additions, resulting in altered C retention in soils due to decomposition and the transference of C from the surface into soils as DOC (Guggenberger 1994, Guggenberger and Zech 1994, Kalbitz et al. 2000, Fenner et al. 2007). Heterotrophic activity is likely to be sensitive to N additions because added N is likely to change C:N ratios of organic matter, increasing sensitivity of microbes (Hobbie and Vitousek 2000, Cusack et al. 2009) and changing community composition of microbes with N addition (Wallenstein et al. 2006). These results suggest that the addition of N to tropical forests has the ability to alter C storage in tropical forests. However, little is still understood as to how soil C storage could be altered by increased levels of nitrogen deposition.

In addition to increases in N availability, changes to global climate due to anthropogenic activities may include warming. Continuing human activities are affecting the Earth's energy budget, with analysis suggesting that there is a small positive energy imbalance on Earth's surface that contributes to the increase in the global heat system (Cubash et al. 2013). As a result, global mean surface temperature over the past 20 years rose at a rate of 0.14 ± 0.06 °C per

decade (Morice et al. 2012). Time series of global temperatures have shown a steady rise most notably since the late 1970s and continuously throughout the 20th century (Foster and Rahmstorf 2011). Climate temperature variability can be explained by El Niño/southern oscillation (Trenberth et al. 2002), volcanic eruptions (IPCC 2007), and solar variations (IPCC 2007, Lean and Rind 2008). Despite improvements in our understanding of anthropogenic activities on global climate, substantial gaps still exist in the data coverage especially in the tropics.

Previous research has identified that organisms living in mid- to high latitudes in the Northern Hemisphere will be most affected by climate warming (Root et al. 2003, IPCC 2007, Parmesan 2007, Rosenzweig et al. 2008) because temperature change is most rapid here (IPCC 2007). However, changes in temperature in tropical regions are likely to affect global circulation.

Evidence shows that recent warming is strongest in the tropics, while El Niño events are also clearly evident, particularly the 1997-1998 El Niño that made 1998 the warmest year (IPCC 2013). Changes in temperature in the tropics can have potential negative effects on global C and nutrient cycling. For example, Wang et al. (2013) found that a 1°C temperature anomaly in the tropics lead to a CO_2 growth rate anomaly with an input of 3.5 ± 0.6 PgC/y. This coupling was explained by increases in heterotrophic respiration and net primary production with temperature variations in tropical ecosystems. This may lead to a negative feedback loop where increasing temperatures are also leading to increased levels of atmospheric CO_2. Coupled with other climate change factors such as enhanced nitrogen deposition and phosphorus increases, changes to global and tropical temperatures are likely to have great impacts on ecosystem structure and functioning.

Although not centrally located as a factor in the global climate change debate, phosphorus plays an equally important role in nutrient cycling and functioning in global and tropical forests. Plant growth and microbial activity are often limited by phosphorous in terrestrial ecosystems (Gusewell 2004, Cleveland and Liptzin 2007, Elser et al. 2007). While reactive nitrogen is naturally produced through biological fixation, phosphorus is a rock derived nutrient that arrives in soil through weathering processes. Although their origins differ, both nitrogen and phosphorus are recycled through the decomposition of soil organic matter. The majority of soil P in early stages of soil development is in primary mineral forms, such as apatite. As apatite weathers, it releases biologically available form of P, such as PO_4^{3-}. Some of this P is taken up by plants and microbes and ultimately released back into the system in inorganic P pools via mineralization or remains in the organic form. During each cycle, P may become sorbed onto secondary soil minerals, precipitated, or leached, and therefore slowly depleting the total available and mineralized P pools in the soil (Walker and Syers 1976).

Being a rock derived nutrient, P limitation often occurs in highly weathered soils, such as those in tropical regions with high turnover rates, that can no longer supply P through weathering (Walker and Syers 1976). Additionally, much of the remaining P is left as insoluble or physically protected, nonlabile P. The release of phosphorus into soils is biologically controlled through the mineralization of soil organic matter by microbes and microbial soil enzymes and physically controlled through sorption/desorption and dissolution/precipitation reactions (Achat et al. 2009, Yang and Post 2011). Elevated CO_2 and warming effects on these biotic and abiotic processes may alter the ratios of N relative to P, which can in turn cause changes to primary productivity, soil organic matter decomposition and biodiversity (Gusewell 2004, Wassen et al. 2005, Liu et al. 2010).

Anthropogenic activities are likely altering the availability of phosphorus in natural ecosystems due to the application of fertilizer and through mining processes. Just as with N fertilization, the application of phosphorus fertilizers was used to keep up with increasing food demand from an increasing population in the 20th century (Brinck 1977, Smil 2000). Phosphorus used for the production of fertilizer is mostly obtained from mined rock phosphate and is often combined with sulfuric acid, nitrogen, and potassium (Cordell et al. 2009). Global use of all P fertilizers reached its peak in 1988 with 16.5 Mt P/year, which was followed was a 25% decline to 12.6 Mt P/year by 1993 due to declining fertilization rates in Europe, Japan, and North America. Overall between 1850 and 2000, the Earth's agricultural soils received about 550 Mt P (Smil 2000). Mining phosphorus for fertilizer is consuming the mineral faster than natural geologic processes can replenish it. The United States may run out of its accessible domestic sources in decades, while few other countries have reserves which could last about a century (Van Kauwenbergh 2003). Changes to phosphorus concentrations and other climate change factors such as nitrogen and warming are likely to have adverse effects on the activity of extracellular enzymes found in tropical forests.

Soil Enzyme Response to Nutrient Additions

Changes in the rate of nitrogen deposition have the potential to affect soil enzyme activities and consequently soil C storage. Early models such as that by Sinsabaugh and Moorhead (1994) predicted that microbes would be limited by C-degrading enzymes and that the addition of nitrogen and phosphorus would result in higher decomposition rates as more resources would be allocated to enzyme production. However, soil microbial enzyme responses to N addition have had opposing results in relation to the direction of change in soil C storage. For example, C pools in early stages of decomposition appear to be more sensitive to N additions

and have shown increased decomposition rates (Berg and Matzner 1997, Neff et al. 2002), which indicates C loss via respiration. This suggests that N availability is a major predictor of the decomposition of organic matter in these forests. In contrast, N deposition has been linked to suppressed decomposition during later stages of decomposition where poor quality C is being decomposed, increasing C storage in long term soil C pools (Carreiro et al. 2000, Swanston et al. 2004, Waldrop and Firestone 2004, Knorr et al. 2005, Olsson et al. 2005). In a study in the eastern United States, N addition decreased the decay rate of high-lignin oak litter (Carreiro et al. 2000) which has been found to lead to increases of C into the soil profile (McDowell and Likens 1988). The initial nutrient litter composition may be a predicting factor affecting these varying responses. For example, a study in northwestern Lower Michigan showed that increased N availability in oak dominated forests resulted in gained soil C, but the sugar-maple dominated forest lost vast amounts of soil C (Waldrop et al. 2004b). It is likely that the oak dominated forest was composed mostly of cellulose while the sugar-maple dominated forest was comprised of lignified compounds. This shows that soil C storage may be responsive to N additions due to differing compositions of soil organic matter and the enzyme communities that are targeting those compounds. One way to better understand the effects of N deposition on decomposition is to focus on the activity of extracellular enzymes which mediate decomposition processes.

Increased nitrogen deposition is likely to alter enzyme activity, resulting in altercations of soil C storage. For example, a study by Waldrop and Zak (2006) in forests of the upper Great Lakes region showed a negative response of phenol oxidase activity to laboratory and field nitrogen additions in forests with high lignin content, while also showing positive responses of phenol oxidase activity in ecosystems low in lignin. These responses are due to an accumulation of organic matter in high lignin forests (Waldrop et al. 2004a, Waldrop et al. 2004b). In these

papers, Waldrop finds that nitrogen deposition increased oxidative activity due to low lignin content of leaf litter and subsequently resulted in a decline in soil organic matter. These studies are consistent with the findings that N additions generally increase rates of C loss for litter that has a relatively low content of lignin, tannin and other secondary plant compounds, while loss of lignified or humified C is generally not altered by additions of N (Fog 1988, Carreiro et al. 2000). This is linked to changes in the distribution and composition of soil microbial extracellular enzyme activity where cellulase and glycosidase activities tend to increase in response to N additions, and oxidative enzymes needed to degrade lignin and humus tend to decrease (Saiya-Cork et al. 2002, Michel and Matzner 2003, DeForest et al. 2004, Gallo et al. 2004). Nitrogen deposition is likely to alter responses from soil enzyme groups and change soil C storage, a potential implication of warming as well.

Temperature Change Effects on Soil Enzyme Activity

Temperature dependence of biochemical cycles, such as decomposition, has been described since the late 19th century (Arrhenius 1889, van't Hoff 1898). Arrhenius introduced scientists to the idea that magnitude of temperature effects on rates are usually too large to be explicable on the basis of how temperature affects translational energies (Laidler 1984). We now know that their assumption of constant temperature sensitivities of respiratory enzymes at all temperatures is incorrect (Lloyd and Taylor 1994, Kirschbaum 2000). The work of Arrhenius (1889) has been expanded to the idea that when substrate availability and enzyme activity are not limiting reaction rates, decomposition rate increases with temperature (Burke et al. 2003, Davidson and Janssens 2006, Friedlingstein et al. 2006). Current models added to this theory with the development that decomposition rates with warming should be greatest at cold temperatures (Del Grosso et al. 2005) Recent models now attempt to understand how

temperature and other environmental factors affect ecosystem processes such as respiration. Enzyme temperature sensitivities are due to the thermal stability of enzyme structures and the sensitivity of catalytic activity (Wallenstein et al. 2011). The structure of a soil enzyme is the main determinant of thermal stability and is defined by primary, secondary, and tertiary elements. The primary structure is the linear amino acid sequence in the secondary structure, where protein folding conformations are created by interactions between amino acid side chains along the polypeptide, and the tertiary structure which is the fully folded states of the globular protein (Wallenstein et al. 2011). The sensitivity of catalytic activity is determined by the accessibility of an active site of the enzyme. The microstability of an enzyme, the energy associated with reversible, local changes in structure, are responsible for the flexibility or rigidity of the active site of an enzyme, which is the location of substrate or ligand binding that leads to catalysis. In a changing environment due to the emission of fossil fuels, our understanding of how enzyme activities will respond to changes in temperature becomes essential to our understanding of soil C storage.

As global average surface temperatures continue to rise (Houghton et al. 2001, Hulme et al. 2002), there is likely to be elevated soil microbial enzyme activity and mobilization of soil nutrients (Freeman et al. 2001). As decomposition activities increase, more CO_2 will be released back into the atmosphere via respiration and a positive feedback loop will likely occur as increased CO_2 will again contribute to more warming (Cox et al. 2000, Prentice et al. 2001, Davidson and Janssens 2006). Peatland studies in the United Kingdom found that phenol oxidase and β-glucosidase enzyme activities tended to increase with higher temperatures (Freeman et al. 2001, Fenner et al. 2007). For phenol oxidase activity an increase in 10 °C resulted in a 36% increase in activity, while for β-glucosidase activity a 27% increase in activity was shown

(Freeman et al. 2001, Fenner et al. 2007). An experiment in tropical forests of Puerto Rico found that in conjunction with N fertilization, soil respiration was sensitive to increased temperature shown with higher Q_{10} values in N fertilized plots in comparison with control plots (Cusack et al. 2010). Enzyme sensitivity to changes in temperature are likely to occur because the activity of these enzymes is controlled by both abiotic (e.g. temperature, water potential, pH) and biotic (e.g. enzyme synthesis and secretion) factors (Burns et al. 2013). Enzyme sensitivity however is not the same in every environment (Luxhoi et al. 2002) and thus the dynamics of C and nitrogen cycling may vary depending on the site specific enzyme responses to changes in temperature. Ultimately at the soil microenvironment scale, changes in enzyme activity due to temperature increases may be relatively small because enzymes are most responsive to substrate availability (Burns et al. 2013).

Most of the recent work looking at the relationship between soil enzyme activity and the response of decomposition rates to temperature has been based in laboratory incubations. This work has shown that increases in temperature are likely correlated with increases in soil enzyme mediated reactions and therefore losses of soil organic matter (Davidson and Janssens 2006, Kirschbaum 2006, Wallenstein et al. 2009, Wallenstein et al. 2011). A study by Bengtson and Bengtsson (2007) found respiration of DOC was limited by the degradation of SOC, which was linked to temperature dependent enzyme activity. Rates of enzyme decomposition of organic matter are determined by the frequency of substrate-enzyme interactions, which are dependent upon the concentration of enzymes and available organic matter within the soil matrix. Temperature affects both the production and turnover of extracellular enzymes in soils, which may indirectly affect the relationship between soil organic matter decomposition and temperature sensitivity (Conant et al. 2011). As enzyme activity increases with temperature, microbes may

decrease the production of enzymes, especially if substrates of other resources are most limiting (Allison et al. 2011).

Temperature sensitivity of extracellular enzymes may also be sensitive to seasonal changes (Koch et al. 2007, Wallenstein et al. 2009). This may be due to trade-offs between enzyme binding abilities and catalytic rates (Hochachka and Somero 2002, Angilletta 2009). This arises because the function of an enzyme requires both an initial conformational change to bind a substrate and then a second conformation change to release the substrate (Conant et al. 2011). These temperature-driven trade-offs can cause changes to membrane composition and thermal adaptation, which may have consequences for soil organic matter turnover (Balser and Wixon 2009, Barcenas-Moreno et al. 2009, Liang et al. 2011, Liang and Balser 2012). Ultimately, thermal responses and adaption in enzyme function can affect whole-scale heterotrophic respiration (Bradford et al. 2008, Bradford et al. 2009, Bradford et al. 2010) and losses of C to the atmosphere.

Hypotheses

We examined microbial community responses to changes in temperature and nutrient additions in six forest sites and two grassland sites in Hawaii. We explored relationships soil C, N, and P to address the following hypotheses:

(1) enzyme activities in sites with high background nutrients and/or C availability are less sensitive to nutrient additions than nutrient poor sites; and

(2) sites poor in background nutrients and/or C show high sensitivity to change in temperature, likely because of a less robust microbial community.

Biophysical Environment

An array of climatic, geological, and biological factors contribute to make the Hawaiian Islands a model ecosystem to study (Vitousek 2004, Chadwick et al. 2007). A model ecosystem is a system which "displays a general process or property of interest, and does so in a way that makes it understandable" (Vitousek 2004). We selected sites that we expected to provide maximum nutrient variation in an attempt to tease out the effects of soil C versus other nutrients versus soil mineralogy. The Hawaii chronosequence represents the best place to be able to control individual ecosystem factors (Figure 54).

The Hawaiian Islands

Climate

Climate accounts for the majority of ecosystem structure and function. The climate of Hawai'i is controlled by its location in the northeast trade wind zone and the ocean that surrounds them. The Hawaiian Islands are located within the tropical Hadley circulation, where solar-heated air rises off the surface near the equator, flows north at high altitude, descends near 30° N and flows back towards the equator. The heated air loses moisture as it comes to the surface, and therefore has very low humidity where it touches down at 30°. The air subsequently picks up moisture from the ocean as it flows back towards the equator (Vitousek 2004).

Temperature and precipitation alone may be more important for determining climate than any other factor. In tropical regions, temperature varies significantly with elevation. Patterns in precipitation and temperature vary drastically throughout the Hawaiian Islands (Mueller-Dombois et al. 1981). Hawaii is characterized by a maritime climate where daily and seasonal temperature variation is moderate. The mean annual air temperature ranges from 24° C at sea

level to 10° C at 2500 m (Atlas of Hawaii 1983), with an environmental lapse rate of ~6.4°C/1000m. The lowest temperature ever recorded in the coastal city of Hilo on the Island of Hawai'i was 11°C while the highest temperature ever recorded was 32°C. The last glacial maximum annual rainfall in the wetter parts of the mountain could have been as much as 50% less than today (Hotchkiss et al. 2000). Soil leaching patterns indicate that high rainfall sites have become adapted to predominating rainfall over the last 10 thousand years and low rainfall sites have been influenced by aridity (Chadwick et al. 2003).

Precipitation is highly variable yearly and winds from the northeast generally range from moist to extremely wet from sea level to 200 m elevation, with an average of 600-800mm/yr. The trade winds are forced upwards on the northeast side of the Hawaiian mountains causing cooling as they rise and resulting in heavy rain on the slopes. Contrastingly, in locations on the Hawaiian mountains that do not reach the tradewind inversion, the wind is deprived of moisture as it passes over the summit. The leeward areas are therefore in a rain shadow, with frequent sunshine and little precipitation. Due to land-sea breeze cycles caused by the Mauna Loa Volcano, eastern Mauna Loa is wet year-round, while the southeast has winter and summer rain, and the northwest is arid (Vitousek 1995). Contrastingly, the southwest side of the islands is generally dry, with as little as 250 mm annual rainfall. The leeward side of Kohala Mountain has mean annual rainfall drops of >3000 mm at the summit to ~150 mm near the coast, a distance of less than 15 km (Vitousek 2004).

Geology

The Hawaiian Islands are created by a plume of hot, buoyant magma that originated deep in the Earth's mantle and pushed through the relatively thin oceanic crust. The islands are a

result of the tectonic movement of the Pacific tectonic plate over this stationary plume that has been active for at least 80 million years. The plume reaches the surface within the Pacific tectonic plate, which is sliding northwest at a rate of 8-9 centimeters per year. Due to this constant movement, the plume has created a chain of volcanic islands, atolls, and submerged seamounts that stretch more than 6000 km from Hawai'i to the Kurile Trench. The plume is now located in the center of the southeastern edge of the Islands, where it sends magma to two active volcanoes on the Island of Hawai'i (Mauna Loa and Kilauea) and a submarine volcano farther to the southeast (Lo'ihi). There have been two active volcanoes (Kualalai on Hawai'i and Kaleakala on Maui) in the last 250 years due to the plume. Plate tectonics cause the plume to move northwest away from the current location resulting in individual volcanoes that are progressively older, both within and between islands. Young Hawaiian volcanoes are shield volcanoes, built up by piles of lava flows. The stratovolcanoes that are located on continental margins are subducted beneath others and produce more viscous lava, more explosive eruptions, and steeper slopes (Vitousek 2004).

During early stages of development, the Hawaiian Islands are derived of theolithic basalt. The initial texture of the parent material ranges from pahoehoe lava flows (smooth, ropy textured, often with large cooling-related cracks) to a'a flows (clinker-type lava) to tephra deposited by lava foundations and the rare explosive eruptions (Vitousek 2004). This creates complex topography and local landscape positions. The mixing of different parent material from eruptions introduces magnesium (Wright and Helz 1987) to the chronosequence caused by variable crystallization of olivine in the volcanoes' plumbing. More than 95% of most Hawaiian volcanoes are derived from theolithic basalt. However, the movement of the islands leads to eruptive activity slows and the magma that does reach the surface spends a longer time in

reservoirs within the volcano. The magma partially crystallizes and fractionates and the resulting material erupts as more alkalic (richer in calcium) (Vitousek 2004).

Soil

We collected soils from two Hawaiian grassland sites that represent a strong gradient in soil mineralogy (Figure 54) (Chadwick et al. 2003) and six forests that vary substantially in parent material age and soil nutrient availability (Figure 54) (Vitousek 2004). Soils younger than 4,100,000 years old are classified as Andisols, while soils older than 4,100,000 are Oxisols. Andisols are influenced by volcanic parent material and byproducts of weathering, while Oxisols are highly weathered soils. Immediately after a volcanic eruption in Hawai'i, the dominant minerals in the lava flow are glass, olivine, clinopyroxene, feldspar, and magnetite-ilmenite. Due to high turnover rates in tropical forests, the minerals weather rapidly particularly after the establishment of plants. Non-crystalline minerals form a highly reactive pedogenic mineral assemblage that persists for more than 1,000,000 years. Relatively unreactive kaolinite and sesquioxide clays accumulate more slowly and dominate older sites (Vitousek 2004).

The Hawaiian forests have rapid organic C and N accumulation in early soil development. Therefore younger soils have considerably more C and N, than older soils. Soil C and N increase until intermediate aged soils (~20,000 years) and then begin to decline in older soils. The increase is due to continued accumulation and stabilization of organic matter by adsorption to the highly reaction non-crystalline minerals that dominate intermediate-aged sites. The Hawaiian grasslands have higher concentrations of C than global mineral soils because of their non-crystalline stabilizing clays (Torn et al. 1997, Chadwick et al. 2003). P has been depleted at dry sites and in wet sites, in most cases less than 50% P remains (Chadwick et al.

2007). The intermediate moisture regime however has higher P remaining in subsurface horizons. This pattern is consistent with plant uptake of nutrients and subsequent surface deposition (Chadwick et al. 2007). Wet sites are particularly nutrient low as these nutrients are lost via leaching as water carries them below rooting depth. Intermediate levels of rainfall result in the most nutrient rich areas.

Vegetation

The isolation of the Hawaiian Islands fosters extraordinary evolutionary distinctiveness, causing a large number of endemic species. However, much of the Hawaiian landscape has been drastically altered by anthropogenic activities. The diverse topography and rainfall patterns on the islands allow for a highly diverse pattern of vegetation. Age is also an important factor contributing to species diversity. Plant species diversity is typically lowest on young soils and greatest in the oldest. The height of the upper canopy increases from the youngest into intermediate-aged soils, and then declines substantially in the older soils. *Metrosideros polyymorpha* is a dominant species, with native tree ferns, *Cibotium glaucum* and *Cibotium chammissois* dominating in the subcanopy in young soils. The main case is that similar forests on different islands consist of representatives of the same genera, although usually not the same species (1994).

Because Hawaiian plant communities evolved on an isolated archipelago, the native flora and fauna are depauperate, and natives have undergone extensive adaptation over a range of habitats (Carlquist 1980). However, recently all but the wettest side of the Kohala Mountains are dominated by exotic pasture grasses (Chadwick et al. 2007). Hawaii has a large transition from forest to savanna ecosystems, which greatly affects species community composition across the

islands. The stark contrast in these ecosystems is the difference in photosynthetic pathways, C_3 being common in forests and C_4 in the savanna. There is a significant continuous shift from C_3 to C_4 with decreasing rainfall (Chadwick et al. 2007).

There are eight main vegetation types across the Hawaiian Islands: (1) strand; (2) coastal; (3) dryland forest and shrub; (4) mixed mesic forest; (5) rain forest; (6) bogs; (7) subalpine woodland, shrubland, and desert; and (8) cliffs. Strand are coastal sites affected by salt spray that are dominated by low shrubs and perennial herbs such as *Scaevola sericea, Jacquemontia ovalifolia, Sesuvium portulacastrum, Sida cordifolia, Vitex rotundifolia, Heliotropium anomalum, H. curassavicum,*and the grass *Sporobolus virginicus*. The coastal zone is not influenced by salt spray and has mesic and arid areas characterized by *Erythrina sandwicensis, Santalum ellipticum, Myoporum sandwicense, Waltheria indica, Sida fallaz,* and *Capparis sandwichiana*. The dryland forest and shrub is severely endangered and comprises 22% of the total indigenous species in Hawai'i with common native species such as *Erythrina sandwicensis, Diospyros sandwicensis, Reynoldsia sandwicensis, Canthium odoratum,* and *Ochrosia compta.* The mixed mesic forest is the most-species rich of the vegetation zones on the island, occurring from 750 to 1,250m. Mixed mesic forests are comprised by *Antidesma platyphyllum, Santalum freycinetianum, Charpentiera* (5 spp.), *Ochrosia* (4 spp.), and *Elaeocrapus bifidus.* The Hawaiian rainforests are characterized by high rainfall with dense vegetation such as *Tetraplasandra* (6 spp.), *Syzygium sandwicensis, Myrsine sandwicensis, Macromitrium owahiense, Adenophorus montanum*, and *Astelia menziesiana*. Hawaiian bogs are located in relatively level montane areas where rainfall exceeds drainage. The vegetation of Hawaiian bogs generally consists of irregular hummocks of cushion-like, low shrubs, sedges, and grasses. The dominant species are *Oreobolus furcatus* and *Rhynchospora lavarum*. Dwarf grass species such

as *Dichanthelium isachnoides, D. cynodon, D. Koolauense,* and *Deschampsia australis* are also common. Subapline woodlands occur at elevations above 1,800 m with a climate that is more temperate than tropical. Parts of the subalpine zone are covered with *Sophora chrysophylla* and *Myoporum sandwicense*, while open shrubland areas are comprise of *Chenopodium oahuense*. Lastly, the cliff habitats largely occur on the windward sides of most of the main islands. They usually consist of almost no soil and spare vegetation such as *Metrosideros polymorpha* and *Vaccinium* spp (1994).

The Hawaiian Islands are also subject to a plethora of successful non-native species. There are relatively 1,200 species of native flora and more than 4,600 known non-native species, of which more than 800 are well established and 90 pose serious threats to native fauna (Chadwick et al. 2007). Non-native species are particularly successful in lowland areas, while higher elevations maintain high levels of native diversity (Smith 1985, Loope and Mueller-Dombois 1989, Wester 1992). These non-native species are likely to change the structure and function of native Hawaiian ecosystems, such as nutrient cycling and communities of soil organisms.

Site Descriptions

We used six forest sites and two grassland sites in Hawaii to examine microbial enzyme community responses to changes in temperature and nutrient additions (Table 2). The six forest sites are comprised of a fertilized and control young forest (Thurston), a nutrient rich young forest (Laupahoehoe), a nutrient rich old forest (Kohala) and a fertilized and control old forest (Koke'e). We paired this with two grasslands sites, a young grassland (Hawi) on the Island of Hawai'i and an old grassland (Kauai) on the Island of Kaua'i.

This study partially makes use of the long-substrate age gradient (LSAG) across the Hawaiian Islands. Forested ecosystems in the LSAG sites range in substrate age from 300 to 4,100,000 years old (Crews et al. 1995, Chadwick et al. 1999, Vitousek 2004). Soil and ecosystem measurements have been made at one site at each age; these sites are matched in elevation, annual precipitation, topography, vegetation, and disturbance history (Vitousek et al. 2009). This allows scientests to assess how nutrient availability affects heterotrophic soil respiration across the soil age gradient (Reed et al. 2011).

i. Field Additions

A full factorial fertilization experiment was established at Laupahoehoe, Thurston, and Koke'e in the late 80's and early 90's of the long substrate age gradient (LSAG) described by Crews et al. (1995). The treatments for Thurston and Koke'e were N (initial application 10 g/m^2 as N, half as urea and half as $(NH_4)_2SO_4$-S are substantial and should not include nutrient limitation), P (initially 5 g/m^2 P as triple superphosphate), and a combination of all other plant macro- and micro-nutrients (designated as "T", receiving 5 b/m^2 of K as K_2SO_4, 5 g/m^2 Ca and 3 g/m^2 of Mg as dolomite, and a commercial micronutrient formulation that added all other essential micronutrients (Granusol #sgb5; 5.61% Mn, 5.12% Zn, 5.36% Cu, 5.38% Mg, 0.54% Bo, and 0.46% Mo)). The initial fertilizer treatments consisted of 25% of the initial application and were repeated at 6 month intervals thereafter. Fertilizer was applied in the interior 15 x 15 m of four 20 m x 20 m plots (Vitousek et al. 1993). This fertilization application was not possible at the Laupahoehoe site due to its size and density of trees. Instead, trees at the Laupahoehoe site were selected ranging from 60-140 cm in diameter, blocked by diameter, and then randomly assigned to a treatment. Fertilizer was spread evenly over a circle 10 m in diameter centered on each tree. The treatments were N (100 $kg \cdot ha^{-1} \cdot y^{-1}$ of N, half as urea and half as ammonium

nitrate), P (100 $kg \cdot ha^{-1} \cdot y^{-1}$ as triple superphosphate) and a complete (minus N and P fertilizer consisting of K (100 $kg \cdot ha^{-1} \cdot y^{-1}$), Ca (100 $kg \cdot ha^{-1} \cdot y^{-1}$), Mg (58$kg \cdot ha^{-1} \cdot y^{-1}$), S (40 $kg \cdot ha^{-1} \cdot y^{-1}$), Fe (8 $kg \cdot ha^{-1} \cdot y^{-1}$), and Mo (0.01 $ka \cdot ha^{-1} \cdot y^{-1}$) designated as "T". There were 8 treatments (control, N, P, T, N+P, N+T, P+T, and N+P+T), with 6 replicates/treatment (Vitousek and Farrington 1997).

Young Forest

The young forest (Thurston) is located at 19°25´ N 155°15´ W on 300 year old soil at 1176m on the Island of Hawai'i in the Volcanoes National Park with an average rainfall of 2,500 mm and an average annual temperature of ~16°C. Thurston contains control and fertilization plots (N x P in a full-factorial design). Soils are relatively infertile with *Mestrosideros polymopha* being a commonly found species. The soil is classified as a Lithic Hapludand (Andisol) and has a poorly development profile that tends towards an Inceptisol (Vitousek 2004). Soils are dominated by primary minerals (olivine, glass, and plagioclase feldspar) derived from volcanic parent material, but these minerals weather out quickly. Primary mineral P (apatite) accounts for most of the soil P. Concentrations of N and P in the *Mestrosideros* foliage were relatively low (Vitousek et al. 2009), while canopy height has a unimodal distribution which suggests an even-aged successional development. Most of the landscape is primary successional forest that dates back to an eruption of the Kilauea Volcano in 1790 (Vitousek 2004). Natural disturbance has affected very little of the landscape, while human disturbance and biological invasions have been quite frequent. Tree growth in fertilized plots was responsive to additions of N alone, suggesting N limitation (Vitousek and Farrington 1997). Contrastingly, litter N additions and N fertilization had no effect on decomposition, regardless of background N limitation (Hobbie and Vitousek 2000).

Nutrient Rich Young Forest

A 20,000 year old site located at 19°33´ N 155°13´ W on the Island of Hawaii at 1170 m, the nutrient rich young forest (Laupahoehoe) has 2,500 mm annual precipitation and an average annual temperature of ~16°C. Laupahoehoe has an increased availability of N and P in soils (Crews et al. 1995, Vitousek et al. 2009). The soils are classified as a Hydric Hapludand(Andisol) (Vitousek 2004) and is dominated by gel-like non-crystalline secondary minerals. Secondary non-crystalline minerals such as ferrihydrite, allophane, and imogolite dominate soils in this nutrient rich young forest. Primary mineral P (apatite) has virtually disappeared from the soil. Foliage concentrations of N and P were also elevated relative to very old and very young sites (Vitousek 1995). Canopy height patterns are relatively trimodal. The Laupahoehoe landscape is older and has turned over many times since first colonization of trees (Vitousek et al. 2009). Forest cover has remained intact by native species, with no evidence of human disturbance and few non-native plants (Vitousek and Farrington 1997).

Nutrient Rich Old Forest

An LSAG site located at 20°03´ N 155°41´ W on the Island of Hawaii at 1122 m, the nutrient rich old forest (Kohala) rests on 150,000 year old soils (Vitousek et al. 2009) that have 2,500 mm annual rainfall and a mean annual average temperature of ~16°C (Riley and Vitousek 1995). The soil is classified as a Hydric Hydrudand (Andisol) and is dominated by gel-like non-crystalline secondary minerals (Vitousek 2004). In the nutrient rich old forest, secondary non-crystalline minerals such as ferrihydrite, allophane, and imogolite dominate soils. Insoluble and/or protected P (occluded P) is the common form of P present. Soil N and P concentrations are relatively rich while foliage concentrations are also elevated (Vitousek 1995). Canopies are

short and height distributions are bimodal. A large portion of the site is native species dominated. 22% of tress are <2 m tall, with large areas dominated by *Dicroanopteris linearis*, *Sphagnum* moss, and short *Metrosideros*. The main form of ecosystem disturbance is not known.

Old Forest

A 4,100,000 year old site located at 22°08´ N 159°37´ W on the Island of Kaua'i at 1134m, this old forest (Koke'e) has low nutrient availability in soils and in foliage (Vitousek et al. 2009). Soils are classified as Plinthic Kandiudox (Oxisol) and tends toward a highly weathered Ultisol (Vitousek 2004). Soils are comprised of secondary kaolin and crystalline sesquioxide minerals that are characteristic of highly weathered tropical forests. Insoluble and/or protected P (occluded P) is the common form of P present and non-occluded P is decreased in the old forest. Koke'e has an average rainfall of 2,500m, an average annual temperature of ~16°C, and mean annual precipitation of 2500 mm per year (Riley and Vitousek 1995). Biological invasions account for 15% of the vegetation, while erosion and deposition make up ~80% of the landscape. Canopies are short and height distributions are bimodal with large portions dominated by native species. 34 % of the target tress are < 2m in height, with large areas dominated by *Dicroanopteris linearis*, *Sphagnum* moss, and short *Metrosideros*. Hurricanes disrupted the ecosystem in 1957, 1982, and 1992 (Herbert et al. 1999). Koke'e contains control and fertilization plots (N x P in a full-factorial design). Tree growth in fertilized plots was responsive to additions of P alone, suggesting P limitation (Vitousek and Farrington 1997). Accordingly, decomposition rates were also enhanced by litter P and increased availability of soil N and P (Hobbie and Vitousek 2000).

Young Grassland

The young grassland (Hawi) site located at 20°05´ N 155°43´W, ranges from 260,000 to 460,000 years old and has an annual precipitation of 270 mm (Chadwick et al. 2003). Mean annual average temperature is ~22°C (Chadwick et al. 2003). Soils are medial-skeletal and mixed in nature. The vegetation is comprised of buffel grass (*Cenchrus ciliaria)* and the keawe tree (*Prosopis pallida*). Soils are located on a 150 thousand year lava flow from Kohala Mountain, Hawaii. Kohala Mountain reaches heights of 1600m into the northeast trade winds and creates an environment with extreme gradients. The ecosystem is greatly impacted on the leeward side of the island by differing evaporation and leaching regimes, and therefore retain more rock-derived nutrients (Chadwick et al. 2003).

Old Grassland

Located at 21°57´ N 159°36´W on the Island of Kaua'i, this old grassland site (Kauai) is positioned on 4,100,000 year old soils at an elevation of 1,200 meters. The grasslands site has an average of 632 mm annual precipitation (Vitousek 1995) and mean average annual temperature of ~18°C. Soils are classified as Oxidsols and tends toward a highly weathered Ultisol (Vitousek 2004). Soils are comprised of secondary kaolin and crystalline sesquioxide minerals that are characteristic of highly weathered tropical forests.

Methods

Overview

This study was conducted in 8 Hawaiian sites expected to provide maximum variation in nutrient availability and background soil C. For each site, twenty soil cores were collected from 0-10 cm depth and subsequently homogenized and sieved. We collected soils from two Hawaiian grassland sites that represented a strong gradient in soil mineralogy (Chadwick et al. 2003). We complemented these sites with soil collected from six forests that vary substantially in parent material age and soil nutrient availability (Vitousek 2004). To test our hypotheses we used laboratory temperature incubations combined with long- and short-term nutrient additions to assess changes in enzyme activities for 8 common soil enzymes that acquire nitrogen, phosphorous, and C from organic matter.

Nutrient Experiment

i. Laboratory Additions

Soils were sieved, pooled by site, and homogenized prior to a 24-hour laboratory incubation with addition of C (sucrose), nitrogen (N) and/or phosphorous (P) in full factorial design. A radiolabeled organic C source (^{14}C-sucrose) was added to soil and respired as $^{14}CO_2$, which was subsequently captured using a liquid base trap. Sucrose was chosen from many reasons, one of which is because it is a simple sugar that contains neither N nor P (Bowman et al. 2004, Meier and Bowman 2008). Samples were split into four groups that received either C alone (400 µg/g C as sucrose), C + N (800 µg N/g dry soil as NH_4MO_3), C + P (800 µg P/g dry soil as KH_2PO_4) or C + N + P additions (800 µg N/g dry soil as NH_4NO_3 and 800 µg P/g dry soil as KH_2PO_4). N and P concentrations were chosen to match levels of addition in the aboveground

fertilization experiment (100 kg/ha/yr of each N and P (Vitousek and Farrington 1997)). Methodology for laboratory additions was derived from Reed et al. (2011) and performed by Reed.

ii. Warming Treatment

We used laboratory 5-20 hour temperature incubations to assess temperature sensitivity of the different soils for 8 common soil enzymes that acquire nitrogen, phosphorous, and C and catalyze oxygen from organic matter. The soils were incubated in the dark at 4, 10, 16, 22, 27, 34, and 40 °C, for which temperatures were chosen based on German et al. (2012). Enzyme Q_{10} kinetics were calculated using:

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

Where [S] is the substrate concentration, K_m is the substrate concentration at half-maximal velocity, and V_{max} is the maximal velocity (German et al. 2012). K_m and V_{max} values were calculated by log transforming the data and performing a non-linear regression (Stone et al. 2012). Q_{10} values were calculated at 10, 20, 30, 34, and 44°C. Three Q_{10} values are reported and analyzed for kinetics:

$$Q_{10\text{-}20} = \frac{Q20}{Q10}$$

$$Q_{20\text{-}30} = \frac{Q30}{Q20}$$

$$Q_{34\text{-}44} = \frac{Q44}{Q34}$$

34 and 44° was selected for the last temperature range because we saw some declines in enzyme response at 34° C.

Enzyme Activity

i. Assay Techniques

Soils were assayed for the activity of eight common enzymes involved in the decomposition of plant litter or cycling of organic C, nitrogen, and phosphorous (Table 2). Enzyme assays were performed at UCLA. Sample suspensions were prepared by adding 2.5 g soil to 100 mL of deionized water. The suspensions were stirred using a magnetic stir plate for 2 minutes each.

Enzymes were all fluoremtric except phenol oxidase and peroxidase. For fluoremetric activity, 100 µl of 200 µM substrate solution (Table 2) and 100 µl soil was added to each sample well. Blank wells received 100 µl substrate and 100 µl deionized water. Background soil wells received 100 µl soil and 100 µl deionized water. Quench standard plates received 100 µl of standard (10 µM 4-methylum-belloiferone) plus 100 µl deionized water. There were eight replicate wells for each blank, background, and quench standard. To stop the reaction, 10 µl of 1.0 M NaOH was added to each well. Fluorescence was measured used a microbial fluoremeter with 364 nm excitation and 450 nm emission filters. After correcting for control and quenching, activity were expressed in units of µmol/(h g) (Sinsabaugh et al. 2003).

Phenol oxidase and peroxidase activities were measured spectrophotometrically using L-3, 4-dihydrozyphenylalanine (L-DOPA) as the substrate. For phenol oxidase, 600 µl of 25 mM L-DOPA and 600 µl soil were added to each sample well. Peroxidase assays received 600 µl of 25 mM L-DOPA plus 10 µl of 0.3% H_2O_2. There were 8 replicate sample wells for each assay.

Activity was measured by analyzing absorbance at 450 nm using a microplate spectrophometer and expressed in units of µmol/(h g) (Sinsabaugh et al. 2003).

Data Processing

Absorbance and fluorescence data was converted to enzyme activity using these formulas.

Formulas

Oxidative enzyme activity (colorimetric)

$$\frac{\frac{ABS_s - avgABS_s - avgABS_b}{200uL} \cdot cal_{slope}}{hours \cdot \frac{g_{dry}}{100mL_{H_2O}} \cdot \frac{1}{2}\, L-DOPAdilution} = \frac{\frac{ABS_s - avgABS_s - avgABS_b}{200uL} \cdot cal_{slope}}{hours \cdot \frac{g_{dry}}{200000uL}}$$

$$= \frac{ABS_w - avgABS_s - avgABS_b}{hour \cdot g_{dry}} \cdot cal_{slope} \cdot 1000$$

Where

ABS_w= soil slurry + L-Dopa absorbance (100 uL soil slurry + 100uL L-Dopa)

averageABS_s= the average of 8 L-Dopa substrate backgrounds (read at same time as soil slurry) (100 uL L-Dopa + 100 uL water)

averageABS_b= the average of soil background (100 uL soil + 100 uLwater)

cal_{slope}=the slope of the calibration curve of L-Dopa developed with mushroom tyrosinase enzyme, plotted as mM L-Dopa versus absorbance

<u>For Soil Moisture Conversion</u>

$$g_{dry} = g_{wet} \cdot \frac{g_{dry}}{g_{wet}}$$

Peroxidative

$$\frac{\frac{ABS_w - avgABS_s - avgABS_b}{200uL} \cdot cal_{slope}}{hours \cdot \frac{g_{dry}}{100mL_{H_2O}} \cdot \frac{1}{2}\,L-DOPAdilution} - avgOXI$$

$$= \frac{\frac{ABS_w - avgABS_s - avgABS_b}{200uL} \cdot cal_{slope}}{hours \cdot \frac{g_{dry}}{200000uL}} - avgOXI$$

$$= (\frac{ABS_w - avgABS_s - avgABS_b}{hour \cdot g_{dry}} \cdot cal_{slope} \cdot 1000) - avgOXI$$

Where

avgABSOXI = the average of 8 final oxidative activity

Time Point Selection

Figures 1-8 display the change in response of oxidative enzyme activity at 5 hours and 17 hours for the nutrient addition experiments. Figures 25-31 display the change in response of oxidative enzyme activity at 5 and 17 hours for the temperature experiment. Final representative time points for oxidative and peroxidative activity were selected as those that had the highest and most representative activity.

Fluorescence

$$\frac{\frac{FLUOR_w - avgFLUOR_s - avgFLUOR_b}{200uL}}{Quench \cdot hours \cdot \frac{g_{dry}}{100mL} \cdot \frac{1}{2}\,L-DOPAdilution} = \frac{\frac{FLUOR_w - avgFLUOR_s - avgFLUOR_b}{200uL}}{Quench \cdot hours \cdot \frac{g_{dry}}{200000uL}}$$

$$= \frac{FLUOR_w - avgFLUOR_s - avgFLUOR_b}{Quench \cdot hours \cdot g_{dry}} \cdot 1000$$

Where

$FLUOR_w$= soil slurry + substrate fluorescence (100 uL soil slurry + 100 uL substrate)

average$FLUOR_s$= the average of 8 substrate backgrounds (100 uL substrate + 100 uL water)

average$FLUOR_b$= the average of soil background (100uL soil slurry + 100uL water)

$$g_{dry} = g_{wet} \cdot \frac{g_{dry}}{g_{wet}}$$

$$Quench = \frac{100uL\ soil\ slurry + 100uL\ 10um\ MUB}{avg(100uL\ pure\ 10um\ MUB + 100uL\ water)}$$

Statistical Analyses

The response of enzyme activity to nutrient additions and warming were analyzed using analysis of variance (ANCOVA). We ran post-hoc Tukey means comparison tests and Fisher's least squares difference tests were employed to test differences in group activity. A fertilization effect was calculated for response variables as the percent change between paired fertilized and control plots for enzyme activities. Analyses were performed using 7.0.2 JMP software (SAS 2007). For ANCOVA, nutrient addition data were averaged by addition (n=2, all values reported as mean ± SE). Means ± 1 s.e. are shown, $p < 0.05$ unless otherwise noted.

Results

Enzyme Activities across Sites

i. Forests versus Grasslands

For laboratory fertilized soils, forests had higher enzyme responses across the board. Oxidative, peroxidative, and C-, N-, and P-acquiring enzymes all had higher activity in forested sites. This is likely a moisture effect not a vegetation effect.

iii. Rainfall Effects

For laboratory fertilized soils, increased precipitation had a positive correlation with oxidative, peroxidative, and C-, N-, and P-acquiring enzyme activities.

Enzyme Responses to Laboratory Nutrient Additions

Oxidative

In an ANCOVA model, relative soil short range order minerals and extractable inorganic N (ug/g-soil) were the only significant predictors. A post-hoc means comparison test showed that high short range order minerals were correlated with elevated oxidative enzyme activity compared to medium and low soil short range order minerals (Figure 17). A regression showed that extractable inorganic N (ug/g-soil) explained 0.7% of the variability (R^2= 0.007861).

Peroxidative

Extractable inorganic N (ug/g-soil) and relative soil short range order minerals were the most significant predictors of peroxidative enzyme activity. Precipitation was also marginally significantly correlated with peroxidative activity (p= 0.0422). A post-hoc regression revealed that extractable inorganic N (ug/g-soil) explained 67% of variability in peroxidative enzyme

activity (R^2=0.6759) and precipitation accounted for 7% of peroxidative activity (R^2=0.0787). A Student's T test showed that higher soil short range order minerals were correlated with enhanced peroxidative activity compared to medium or low short range order minerals (Figure 18).

Alpha-Glucosidase

Lab treatment and extractable inorganic N (ug/g-soil) were extremely significant predictors of Alpha-glucosidase activity. C:N ratio (p=0.0034) and precipitation (p=0.001) were also significant predictors of Alpha-glucosidase activity. Relative short range order minerals were significantly correlated with Alpha-glucosidase activity (p=0.0144). A post-hoc means comparison showed that +CNP and +CN was correlated with significantly higher alpha-glucosidase activity compared to +CP and +C and that higher and medium short range order minerals were correlated with higher activity as compared to low short range order minerals (Figure 19). A fitted regression showed that extractable inorganic N (ug/g-soil) explained 44% (R^2=0.4428), C:N ratio explained 11% (R^2=0.1114), and precipitation explained 10% (R^2=0.1070) of the variability in Alpha-glucosidase activity.

Beta-Glucosidase

Total C (%), total N (%), extractable inorganic N (ug/g-soil), soil short range order minerals, vegetation, and lab treatment as predictors was significant. There is a trend with vegetation type (p=0.06), but precipitation was not significant. A post-hoc means comparison test showed that Beta-glucosidase activity was higher in +CNP and +CN treatments as compared to +C and +CP treatments (Figure 20).

Cellobiose

An initial ANCOVA model showed that lab treatment, C:N ratio, and extractable inorganic N (ug/g-soil) were all highly significant predictors of cellobiose enzyme activity. Vegetation was also a significant predictor (p=0.0051). A post-hoc means comparison test showed that +CN and +CNP was correlated with significantly higher cellobiose activity in relation to +C and +CP (Figure 21). A regression showed that both C:N ratio and extractable inorganic N (ug/g-soil) explained 33% of the variability in bellobiose activity (p= 0.3359 and p=0.3347 respectively).

Xylosidase

Lab treatment, total N (%), C:N ratio, and vegetation were all highly significant predictors of xylosidase enzyme activity. Interestingly, a post-hoc test revealed that lab treatments did not significantly differ from one another (Figure 22). A fitted regression showed that total N (%) explained 87% of the variability in xylosidase activity (R^2=0.8714).

N-Acetylglucosamine

Initial ANCOVA models indicated that lab treatment and total N (%) were both significant. ANCOVA for Nagase showed that a model including only Total N (%) and lab treatment was significant. A post-hoc regression showed that %N explained 50% of the variability in Nagase activity and that nagase activity was significantly correlated with total N (%) (R^2=0.50). A Least Significant Difference Test showed that +CN and +CNP treatments had significantly higher Nagase activity relative to +C and +CP treatments (Figure 23).

Phophatase

Lab treatment, extractable inorganic N (ug/g-soil), Bray's P (ug/g-soil), relative soil short range order minerals, and precipitation were all highly significant predictors of phosphatase activity. Total C (%) was also a significant predictor. Interestingly, a post-hoc test showed that +CN was correlated with significantly higher phosphatase enzyme activity relative to +CNP, +CP, and +C (Figure 24).

Effects of Laboratory Warming: Temperature Response and Kinetics

Temperature Response

Oxidative

Initial ANCOVA models indicated that total C (%), total N (%), extractable inorganic N ug/g-soil), and relative soil short range order minerals were all highly significant predictors of oxidative Q_{10}. A post-hoc regression should that total N (%) explained 1.4% of the variability, while extractable inorganic N explained 0.7%. A Student's T means comparison test showed that high and low soil short range order minerals were correlated with a higher oxidative Q_{10} response when compared to moderate minerals (Figure 40).

Peroxidative

Relative short range order minerals were the only significant predictor of peroxidative Q_{10} sensitivity. Extractable inorganic N (ug/g-soil) was a marginal predictor (p=0.0607). A post-hoc Student's T means comparison test showed that moderate short range order minerals corresponded with a significantly higher peroxidative Q_{10} sensitivity as compared to high and low minerals (Figure 41).

Alpha-Glucosidase

Total C (%), total N (%), extractable inorganic N (ug/g-soil), Bray's P (ug/g-soil), and relative soil short range order minerals were all significant predictors of alpha-glucosidase Q_{10}. A post-hoc regression showed that total C (%) explained 36.84% of the variability in alpha-glucosidase Q_{10} while total N (%) explained 38.71%. a Student's T means comparison test showed that low and moderate level short range order minerals had higher alpha-glucosidase Q_{10} sensitivity than high minerals (Figure 42).

Beta-Glucosidase

Total N (%) and extractable inorganic N (ug/g-soil) were significant predictors of beta-glucosidase Q_{10} sensitivity. Relative short range order minerals were marginally significant ($p=0.0583$). Total N (%) explained 3% of the variability.

Cellobiose

Total N (%), extractable inorganic N (ug/g-soil), Bray's P (ug/g-soil) and relative short range order minerals were all significant predictors of cellobiose Q_{10}. A post-hoc regression showed total N (%) explained 36.9% of the variability in Q_{10} extractable inorganic N explained 8.5% and Bray's P explained 12.3%. Student's T means comparison showed that low soil short range order minerals corresponded with higher cellobiose Q10 in comparison to high and moderate minerals (Figure 43).

Xylosidase

Total C (%), total N (%), extractable inorganic N (ug/g-soil), Pray's P (ug/g-soil) and relative soil short ranger order minerals were all significant predictors of xylosidase Q_{10}. A post-

hoc regression showed that total C (%) explained 45.4% of the variability, total N (%) explained 41.5% and Bray's P explained 14.5%. Student's T means comparison showed that low soil short range order minerals corresponded with higher xylosidase Q10 in comparison to high and moderate minerals (Figure 44).

N-Acetylglucosamine

Total C (%), total N (%), extractable inorganic N (ug/g-soil), and relative soil short range order minerals were all significant predictors of n-acteylglucosamine Q_{10}. Bray's P (ug/g-soil) was marginally significant (p=0.0554). A post-hoc regression showed that toal C (%) explained 23.9 % of the variability while total N (%) explained 23.8%. Student's T means comparison showed that low soil short range order minerals corresponded with higher n-acetylglucosamine Q10 in comparison to high and moderate minerals (Figure 45).

Phophatase

Total N (%) was the only significant predictor of phosphatase Q_{10} sensitivity. Total C (%) was a marginally significant predictor (p=0.08). A post-hoc regression showed that total N explained 13.2% of the variability in phosphatase Q_{10}.

Kinetics

For oxidative kinetics, $Q_{10\text{-}20}$ did not significantly differ from $Q_{20\text{-}30}$ which did not significantly differ from $Q_{34\text{-}44}$, yet $Q_{10\text{-}20}$ significantly differed from $Q_{34\text{-}44}$ (Figure 46). For peroxidative kinetics, $Q_{10\text{-}20}$ was significantly higher than $Q_{20\text{-}30}$ and $Q_{34\text{-}44}$ (Figure 47). For alpha-glucosidase Q_{10} sensitivity, Q10-20 was significantly higher than $Q_{20\text{-}30}$ which was significantly higher than $Q_{34\text{-}44}$ (Figure 48). For beta-glucosidase kinetics, $Q_{10\text{-}20}$ and $Q_{20\text{-}30}$ were

significantly higher than $Q_{34\text{-}44}$ (Figure 49). Cellobiose kinetics showed that $Q_{10\text{-}20}$ was significalty higher than $Q_{20\text{-}30}$ and $Q_{34\text{-}44}$ (Figure 50). For xylosidase Q10 sensitivity, $Q_{10\text{-}20}$ was significantly higher than $Q_{20\text{-}30}$ and $Q_{34\text{-}44}$ (Figure 51) For nagase kinetics, $Q_{10\text{-}20}$ did not significantly differ from $Q_{20\text{-}30}$ which did not significantly differ from $Q_{34\text{-}44}$, yet $Q_{10\text{-}20}$ significantly differed from $Q_{34\text{-}44}$ (Figure 52). $Q_{10\text{-}20}$, $Q_{20\text{-}30}$, and $Q_{34\text{-}44}$ did not significantly differ from each other for phosphatase kinetics (Figure 53). .

Discussion

Implications for Decomposer Activity with Global Change in Nutrient Availability

In support of our first hypothesis, enzyme activities in sites with high background nutrients and/or C availability were often less sensitive to nutrient additions than nutrient poor sites. Additions of +CN and +CNP corresponded with higher C-, N-, and P- acquiring enzyme activities, with lab treatment being a significant predictor for all hydrolytic enzyme activities. For, C-, N-, and P- acquiring enzymes, sites high in background nutrients (Laupahoehoe and Kohala) were most commonly the least responsive to additions of +CN and +CNP, while nutrient poor sites (Thurston Control and Koke'e Control) most often showed the highest response relatively. This agrees with the theory that enzyme production and activity should be highest when nutrients are scarce. Since enzyme production is energy intensive, microbes will only spend energy on the growth and metabolism required to produce enzymes when background nutrients are hard to find (Koch 1985). Harder and Dijkhuizen (1983) found that when available nutrients are scarce, microbes produce soil enzymes to release nutrients from complex chemical sources. Additionally, in a study in constructed wetlands in Mid-Wales, the addition of carbon stimulated cellulase activities (Shackle et al. 2000). Chrost (1991) found that the addition of phosphorus and cellobiose resulted in an increase of phosphatase and β-glucosidase production in aquatic ecosystems. Conversely, Allison and Vitousek (2005) found that adding a complex substrate alone had no effect on enzyme activity but adding simple and complex resources in combination increased activity. This study indicated that the addition of simple and complex nutrients together, ammonium and phosphate with cellulose, caused a C limitation relative to N and P, creating an environment where microbes want to invest in C acquisition activities such as

the production of soil enzymes. These responses suggest that enzyme production and activity may be responsive to the presence of substrates when background nutrient status is low.

Interestingly, laboratory additions did not significantly predict oxidative or peroxidative enzyme activities. Similarly, a study by Carreiro et al. (2000) found that added N is likely to be correlated with oxidative enzymes and actually decrease activity. In fact, many studies have found that increased N availability has not been shown to be correlated with increased oxidative activity (Kalbitz et al. 2000, DeForest et al. 2005, Cusack et al. 2010). Our study found that oxidative and peroxidative enzymes activities were elevated when there were high background soil short range order minerals as compared to medium and low. We hypothesize this is due to the fact that oxidative and peroxidative enzymes can be stabilized on short range order minerals. In a study on volcanic soil with and without added enzymes, the addition of mineral allophane had a strong positive effect on enzyme activity and the addition of mineral ferrihydrite had a weak positive effect on enzyme activity, while the addition of humic acids was strongly correlated with decreases in enzyme activity (Allison 2006). This suggests that minerals can enhance potential enzyme activity, agreeing with results found in our study which revealed that high short range order minerals were correlated with higher oxidative and peroxidative activity. Non-crystalline minerals such as allophane and ferrihydrite are common on soils derived from volcanic parent material (Vitousek et al. 1997), such as those in Hawaii. Because of the complex nature of allophane and ferrihydrite, they can act as stabilizers for soil proteins, such as enzymes (Wada et al. 1979, Schwertmann and Taylor 1989). Thus, soil minerals may help to stabilize oxidative and peroxidative enzyme activity, which can lead to increases in enzyme activity.

Implications for Decomposer Activity with Climate Change

It has long been known that extracellular enzyme activities are sensitive to changes in temperature (Lloyd and Taylor 1994, Koch et al. 2007, Wallenstein et al. 2009, Stone et al. 2012). However, local soil microbial extracellular enzyme responses to different temperature regimes are not well understood. In contrast to our hypothesis, enzyme temperature responses, measured as Q_{10} values, were strongest for soils from relatively nutrient- and C-rich forests, and lowest for drier sites poorer in soil nutrients and C. A nutrient rich forest site (Laupahoehoe) showed the most dramatic response to changes in temperature for all C-, N-, and P- acquiring enzymes. Overall in our study, increases in temperature were associated with higher enzyme activities for oxidative, and N- and P-acquiring enzymes. This finding is consistent with many others, that warming temperatures correspond with increased soil enzyme activities (Lloyd and Taylor 1994, Koch et al. 2007). Interestingly, C-acquiring enzymes alpha-glucosidase and beta-glucosidase showed decreases in enzyme activity from 34-40 °C (Figure 34 and Figure 35 respectively), while cellobiose, xylosidase , N-acteylglucosamine, and phosphatase activity increased continuously (Figure 36-39 respectively). Warming temperatures generally increase forest productivity, but only until an optimum temperature is reached for which processes show a drop in activity if the temperature increases further (Lukac and Godbold 2011). This suggests that enzyme responses reach a threshold of ultimate temperature until increasing temperatures have negative effects.

Local adaptation of soil enzymes may also play a role in temperature sensitivities. This study found that enzyme activities showed faster increases in Q_{10} temperature ranges of 10-20 °C as opposed to 20-30 °C and 34-44 °C. The temperature sensitivity of enzymes may be related to 'local adaptation' (Belotte et al. 2003) of organisms to a specific temperature regime across

season or latitude. This is likely due to the production of different isoenzymes (enzymes that differ in amino acid sequence but catalyze the same chemical reaction) by the same microbes by transcribing alternate genes (Loveland et al. 1994). Increasing the catalytic potential of an enzyme is the most common adaptation for offsetting the decrease in enzyme activity (Georlette et al. 2004). These enzymes have a high specific activity at a specific temperature at which the maximum activity occurs. The high activity that is achieved at temperatures similar to background conditions is achieved through small modifications in stabilizing factors that make the enzyme structurally flexible (Feller and Gerday 2003). This flexibility allows greater access to the active site and reduced enzyme specificity (Feller 2003). By altering substrate affinity at high temperatures, enzyme activity is likely to decrease (Wallenstein et al. 2009). This theory is in agreement with our findings, that increasing temperatures causes a decrease in Q_{10} values.

We observed that C-acquiring enzymes had higher Q_{10} values than N-acquiring and P-acquiring enzymes (Table 7). In our study, N- and P- acquiring values had lower Q_{10} (overall mean of 1.10 and 1.06 respectively) than C-acquiring enzymes (overall mean of 1.17). This simple observation that different enzymes have different temperature sensitivities may have huge implications for global climate change and soil structure and function. Without changes to the enzyme pool, the relative activity of these enzymes can change along with temperature, resulting in high C-mineralization relative to N- and P- mineralization. Wallenstein et al. (2009) also found that N-degrading enzymes had lower Q_{10} values (overall mean of 1.59) than C-degrading enzymes (overall mean of 2.07). This is consistent with findings in alpine tundra soils that C-mineralization is more temperature sensitive than N-mineralization (Koch et al. 2007). This may result in increased mobilization of C and N-limitation, which was also observed in Arctic soils in summer (Schimel and Bennett 2004).

The relative rate at which temperature is changing is higher in temperate regions than it is in the tropics, so enzymes will have a harder time adapting to these new conditions that are expected to arise with global climate change. A unifying theory in enzyme studies is that cold-adapted enzymes tend to be more sensitive to increasing temperatures, likely to occur with increasing global climate change factors, than warm-adapted enzymes (Somero 2004, Koch et al. 2007, Dong and Somero 2009). Since tropical enzymes are more evolved to deal with warmer temperatures, increases in temperature due to climate change may not be as destructive to tropically derived enzymes as for temperate enzymes.

Implications for Soil C Storage

Taken together, responses of oxidative, C-, N- and P- acquiring enzyme activity to changes in warming and nutrient additions have implications for soil organic matter decomposition and soil nutrient cycling in future climates. Soil C storage is partially controlled by decomposition processes and our understanding of their responses to changes in climate is therefore very important. In this study, increases in N availability were shown to increase hydrolytic enzyme activities, but have no significant effect on oxidative enzymes. The positive response of hydrolytic enzyme activity to N availability has also been seen in many other controlled experiments in N-rich tropical forests (Hobbie and Vitousek 2000, Allison and Vitousek 2004, Cusack et al. 2011). In addition, the suppression of oxidative enzyme activities to N fertilization is well known (Keyser et al. 1978, Kirk and Farrell 1987, Fog 1988). These responses are likely to result in the loss of simple carbon compounds such as cellulose back into the atmosphere as CO_2 and the storage of carbon from lignin compounds (Sinsabaugh et al. 2002).

The positive response of hydrolitic enzyme activities to warming will likely negatively affect decomposition of soil simple C compounds and soil C storage. This agrees with many studies that found that when substrate availability is not limiting, enzyme activity increases with temperature (Burke et al. 2003, Davidson et al. 2006, Friedlingstein et al. 2006). Organic matter decomposition is expected to increase with short-term temperature rise due to the fact that enzyme reactions are sensitive to warmer temperature and therefore increase sharply (Davidson et al. 2006, Kirschbaum 2006, Lawrence et al. 2009, Wallenstein et al. 2009, Wallenstein et al. 2011). When organic matter is physically accessible it is quickly degraded by soil enzymes. These enzymes are produced by microbes, which can only assimilate soluble, low molecular weight compounds, to break down most of the constituents of soil organic matter. The degradation of the lowest quality soil organic matter, which are targeted by hydrolytic enzymes, are likely to be the most temperature sensitive (Conant et al. 2011). This explains why hydrolytic enzymes were most sensitive to changes in temperature, while oxidative enzymes did not show this sensitivity. Soil enzymes act as gatekeepers for terrestrial carbon fluxes, either by causing carbon release to the atmosphere through decomposition activity or preventing its release through stabilization (Liang and Balser 2012). Therefore, if warming temperatures are causing increasing enzyme activity and decomposition, more CO_2 may be emitted to the atmosphere. The largest source of CO_2 emission is heterotrophic respiration of soil organic matter (Prentice et al. 2001, Davidson et al. 2006). Increasing temperatures may therefore be decreasing the storage of simple C compounds in the soil as hydrolytic enzyme activity increases.

Appendices

Figure 1. Hawi oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

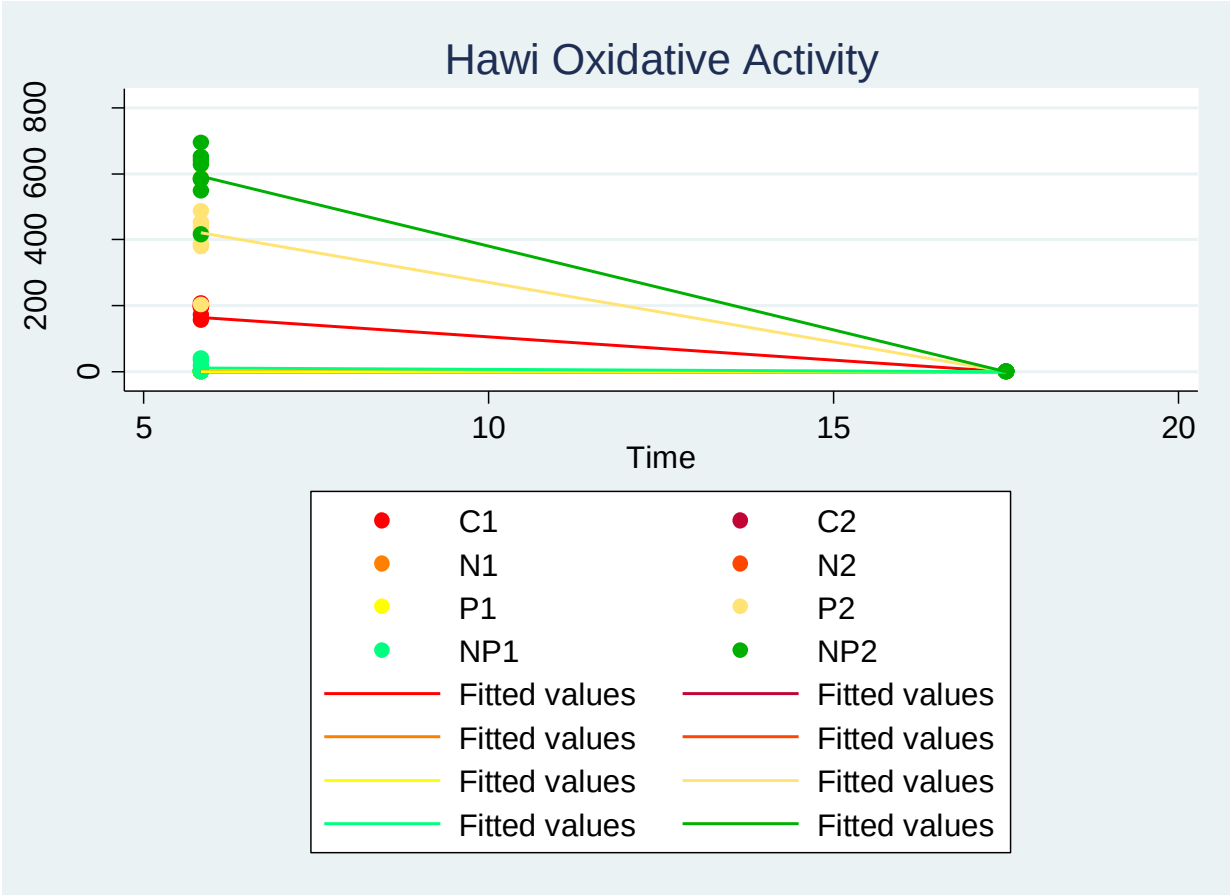


Figure 2. Kauai oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

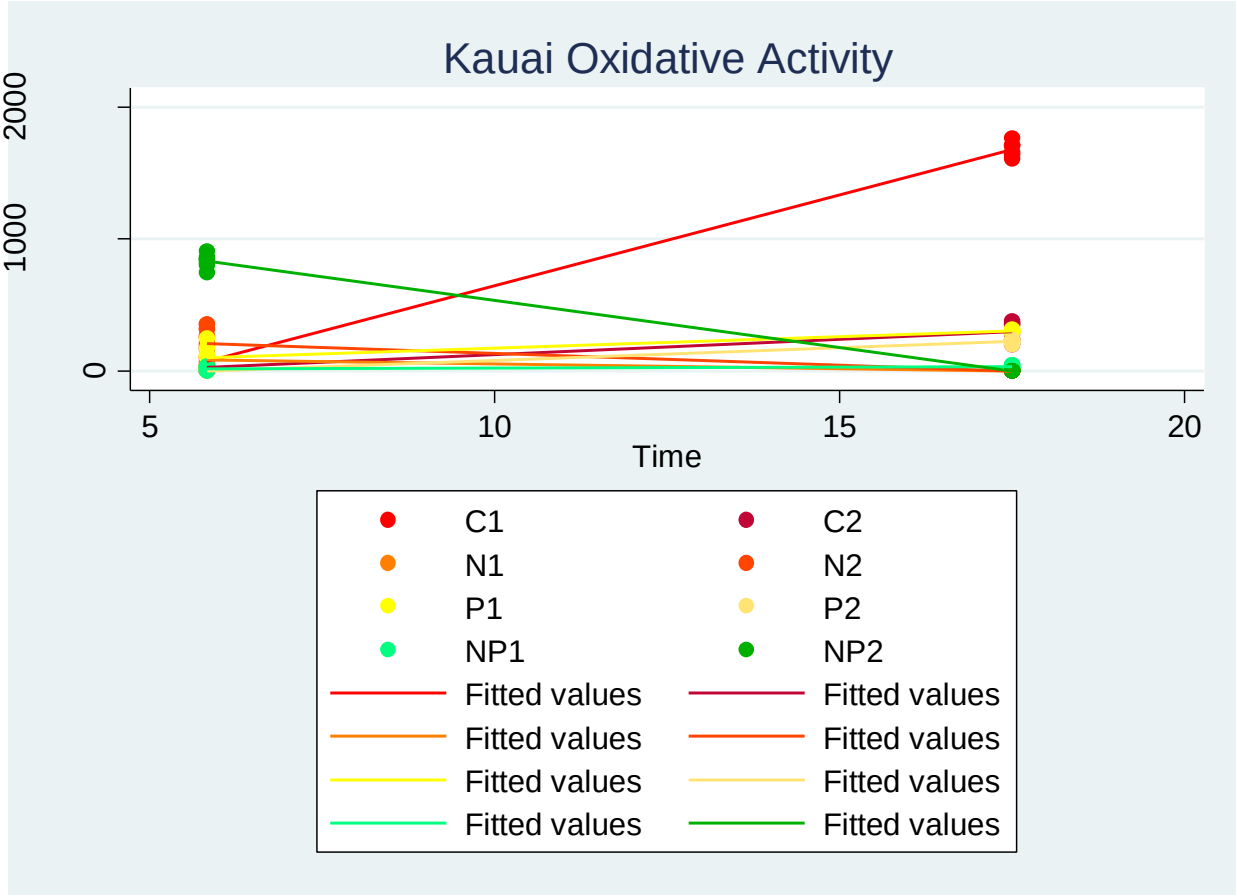


Figure 3. Kohala oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

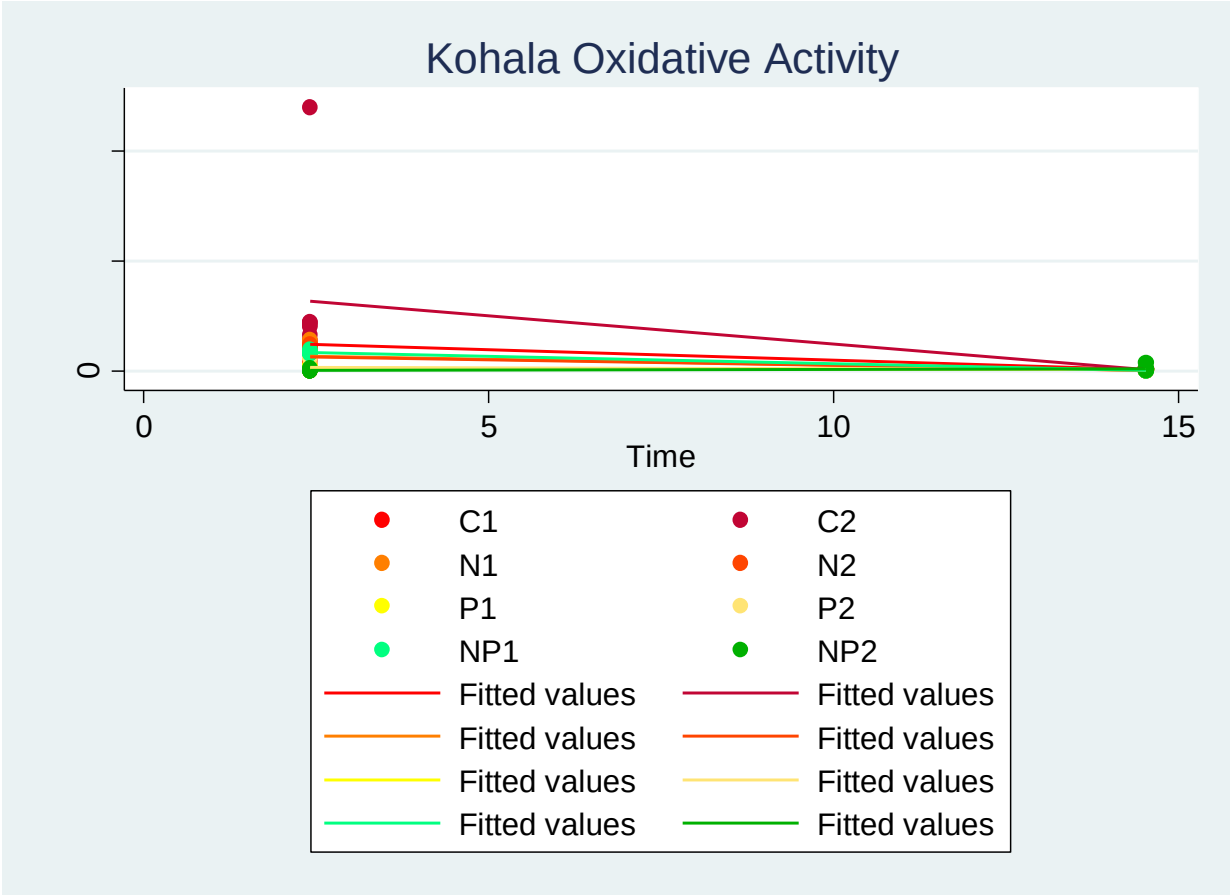


Figure 4. Koke'e Control oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

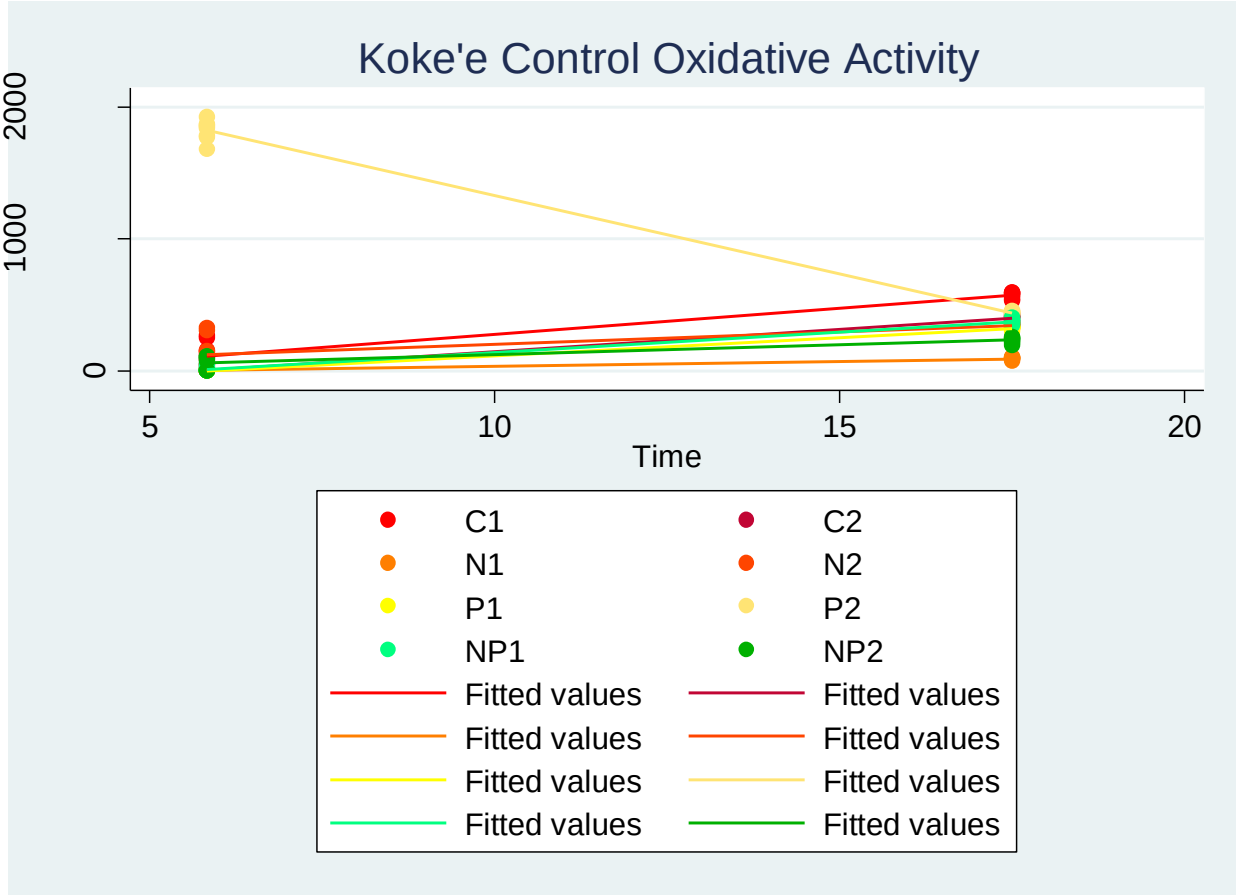


Figure 5. Koke'e +NP oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

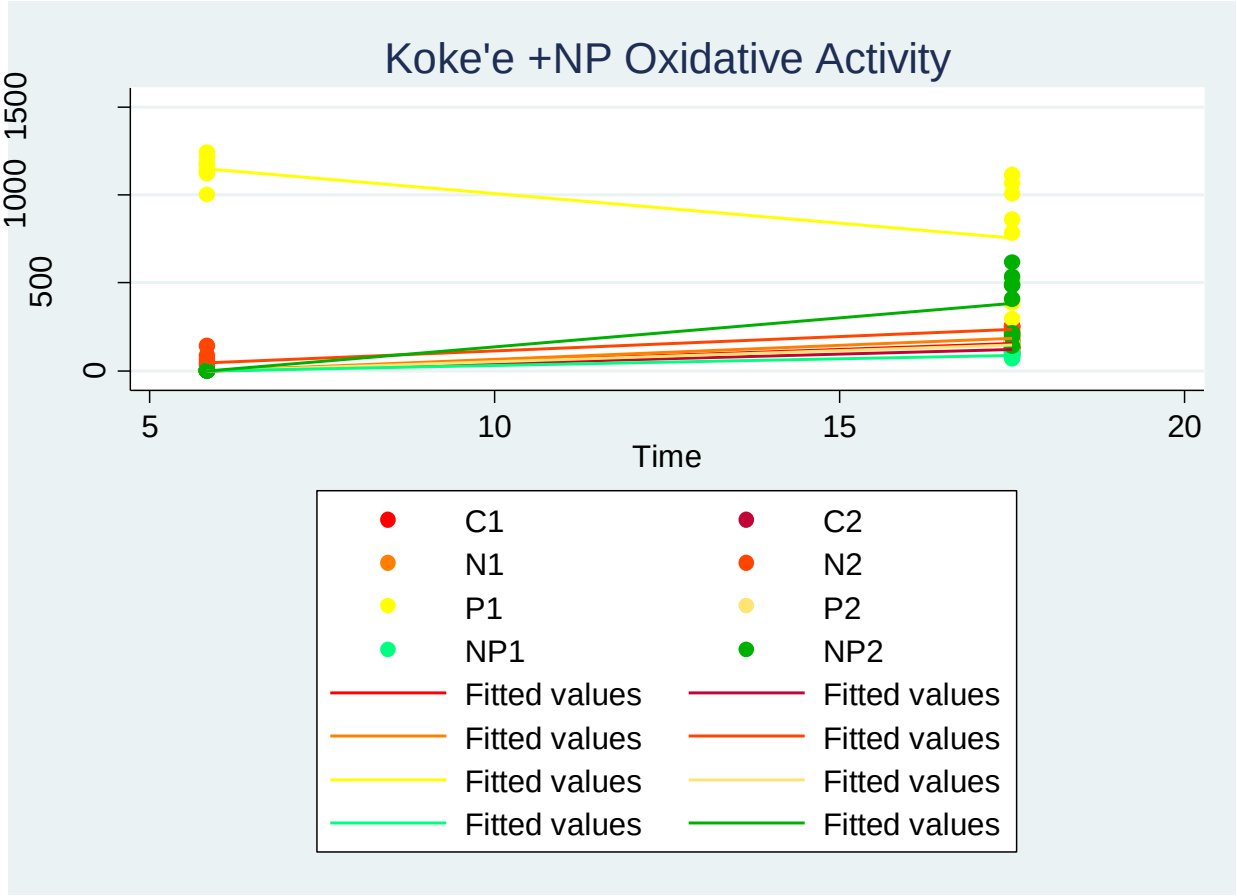


Figure 6. Laupahoehoe oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

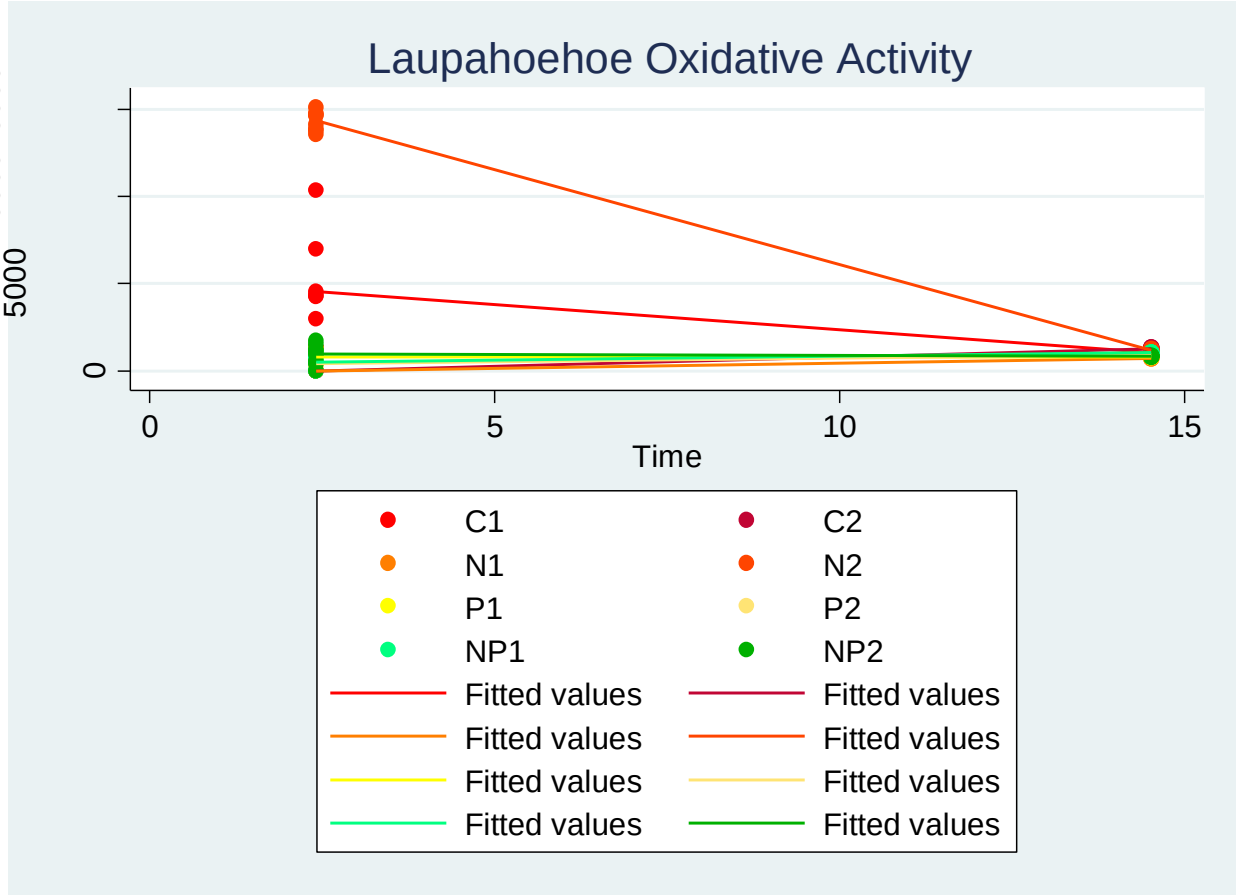


Figure 7. Thurston Control oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

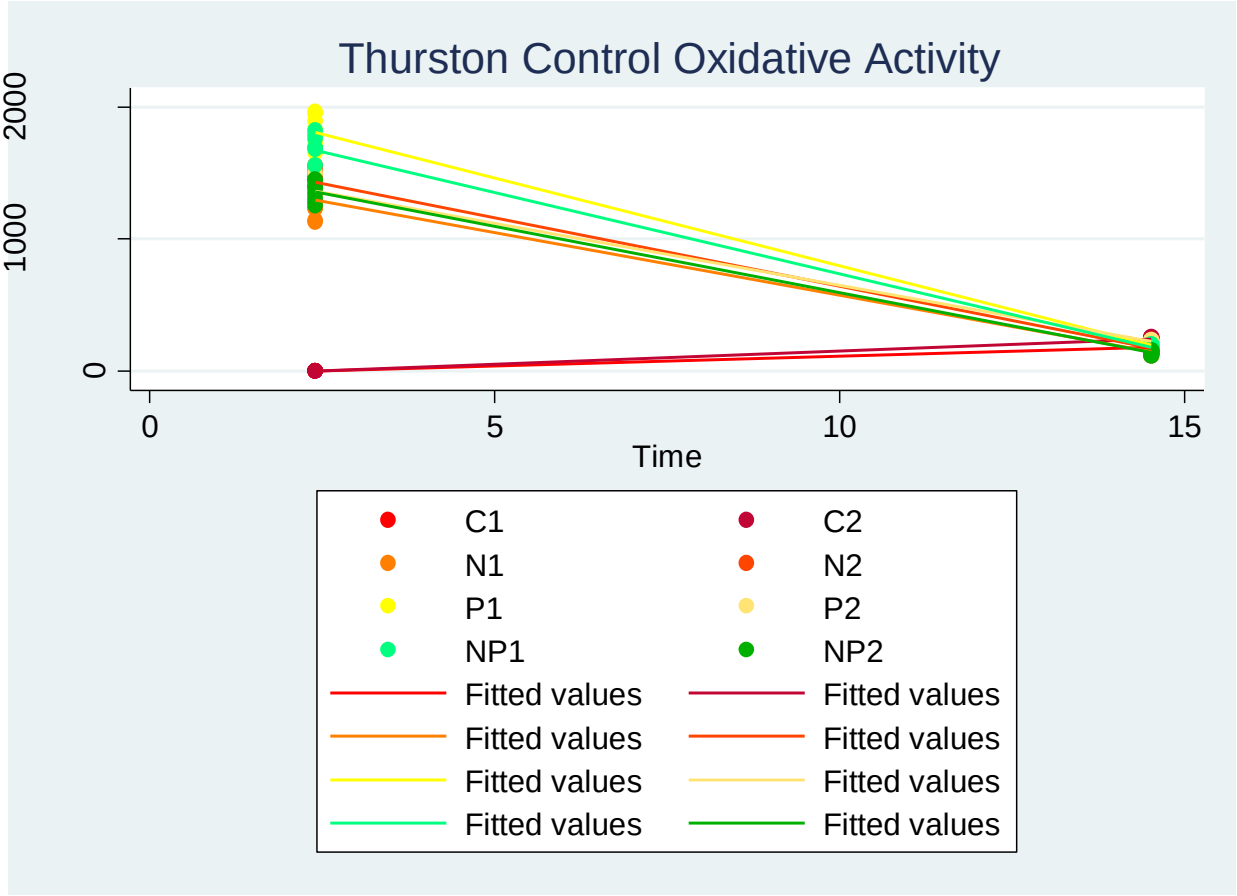


Figure 8. Thurston +NP oxidative enzyme activity response to nutrient fertilization over two time points (~ 6 hours and ~ 17 hours).

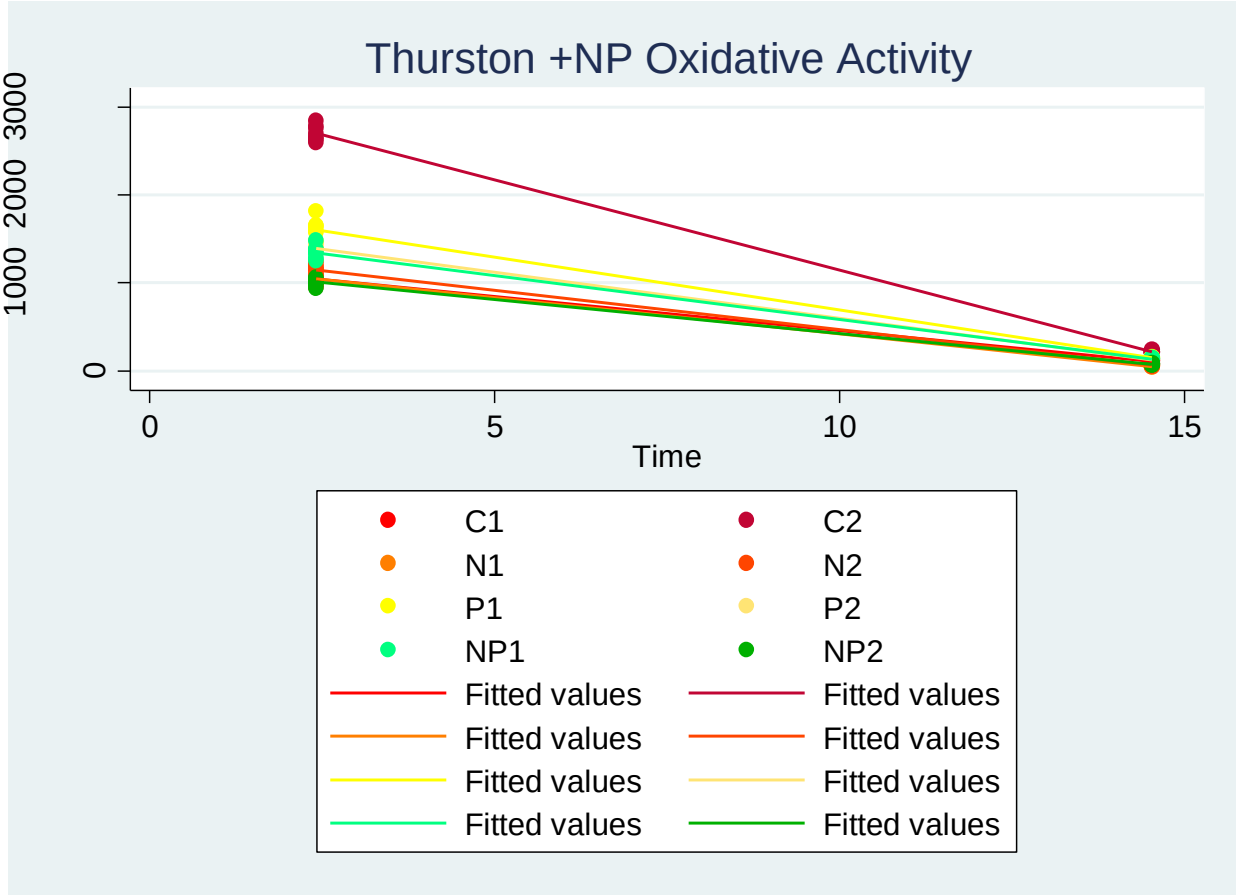


Figure 9. Response of log oxidative enzyme activity to nutrient additions.

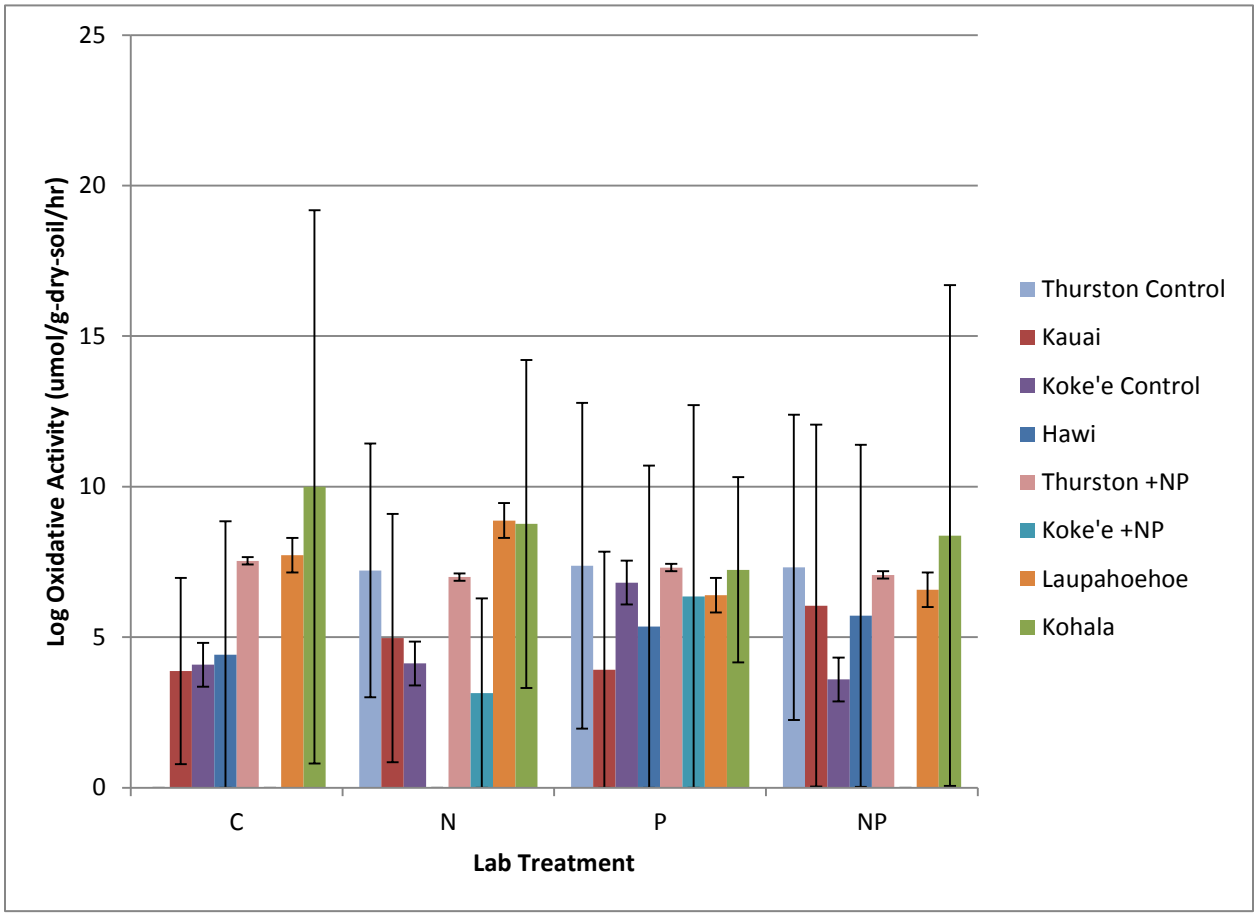


Figure 10. Response of peroxidative enzyme activity to nutrient additions.

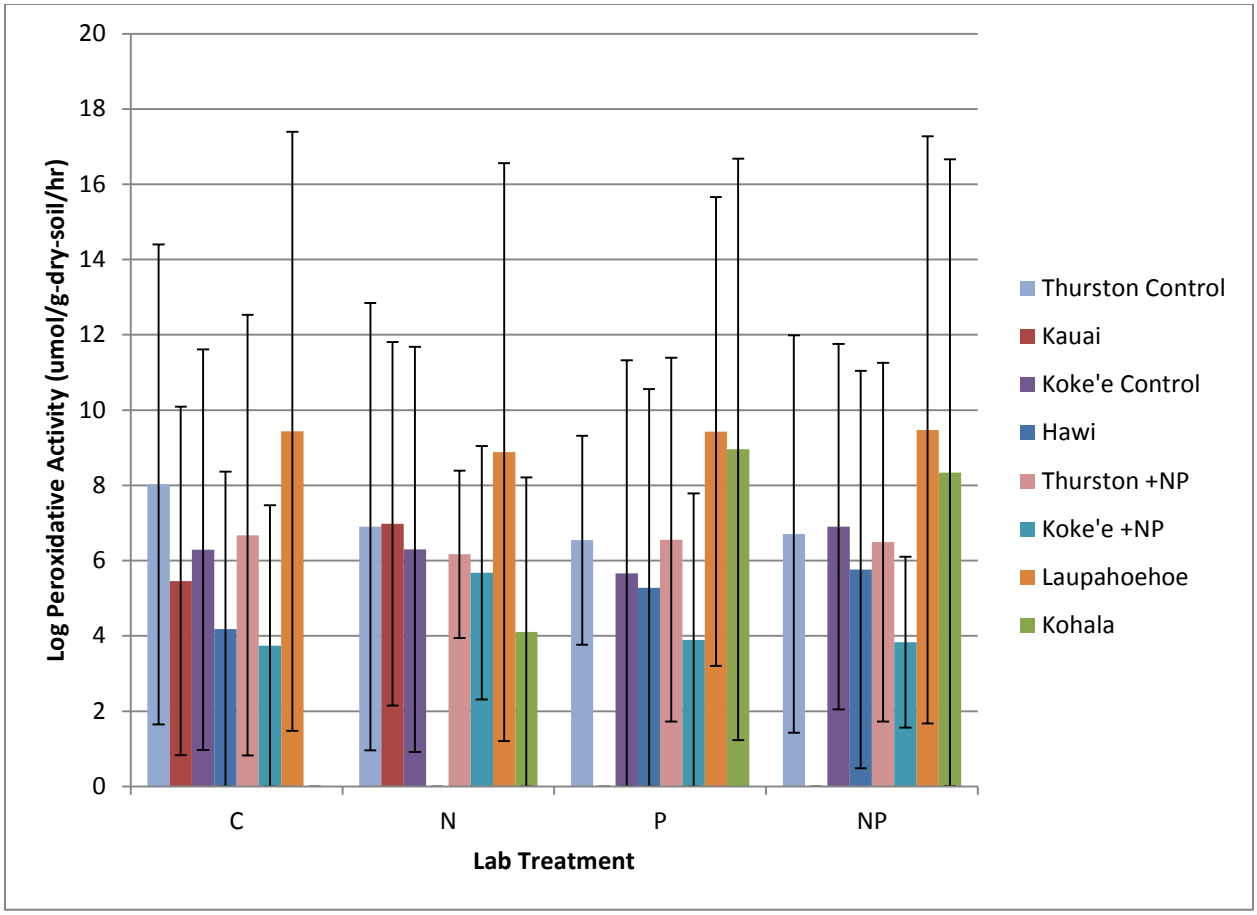


Figure 11. Response of alpha-glucosidase enzyme activity to nutrient additions.

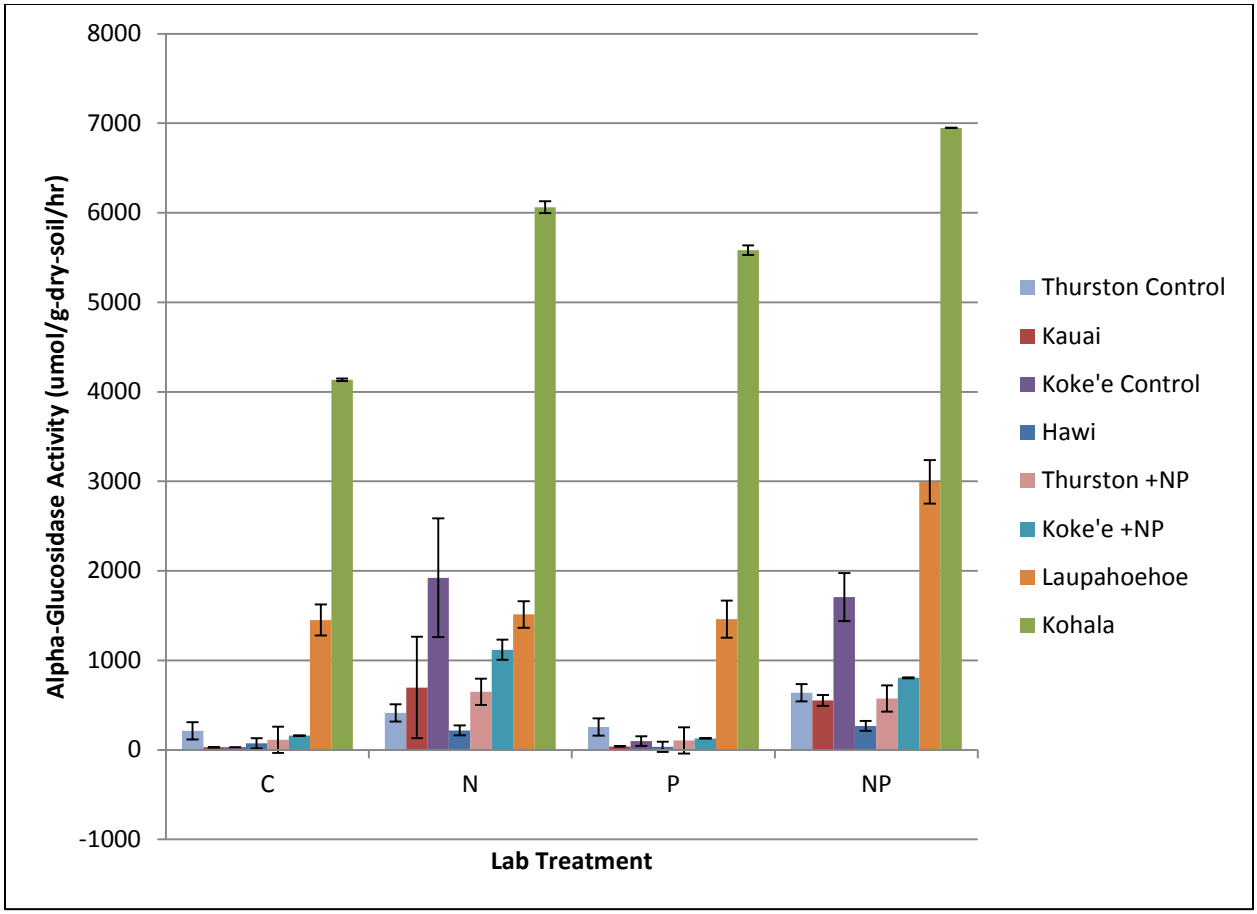


Figure 12. Response of beta-glucosidase enzyme activity to nutrient additions.

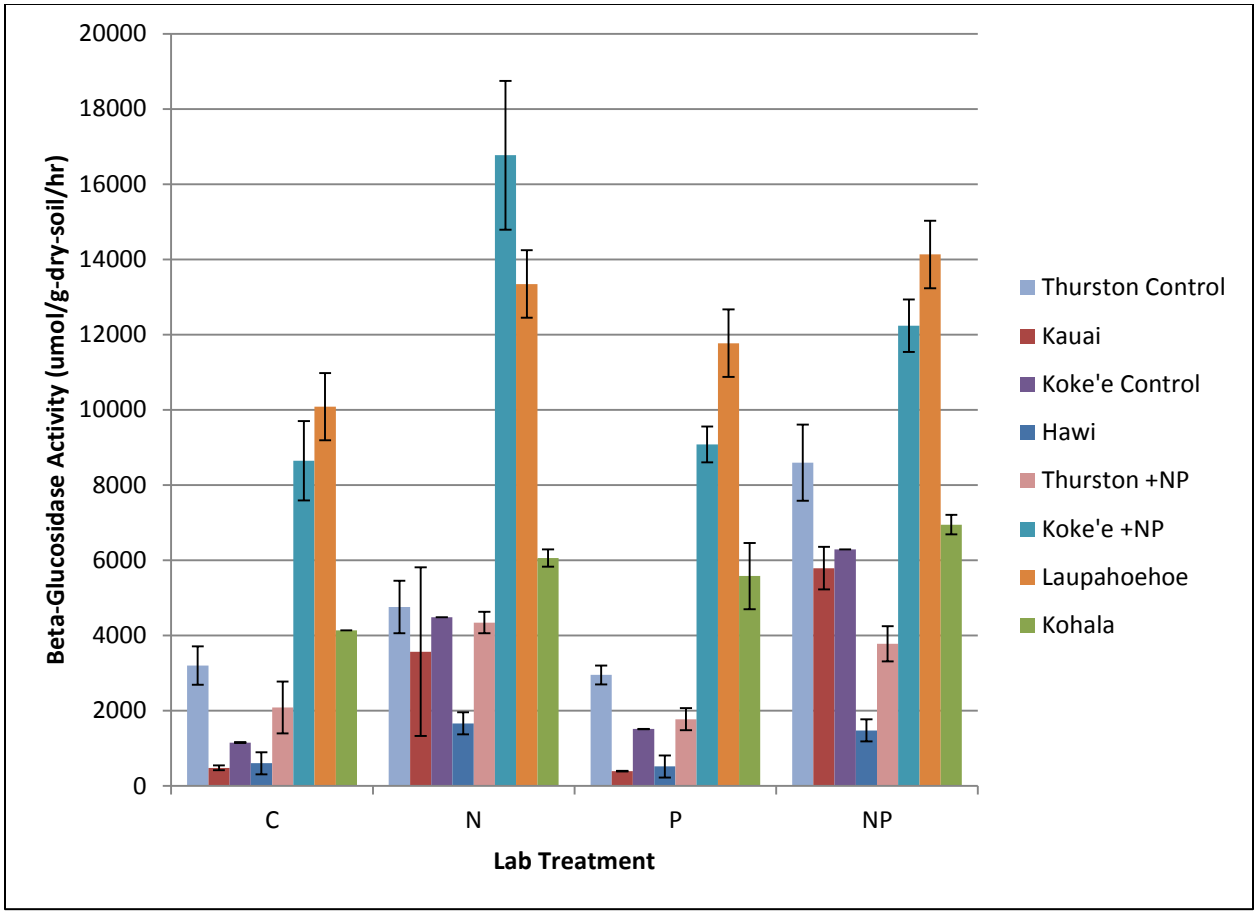


Figure 13. Response of cellobiose enzyme activity to nutrient additions.

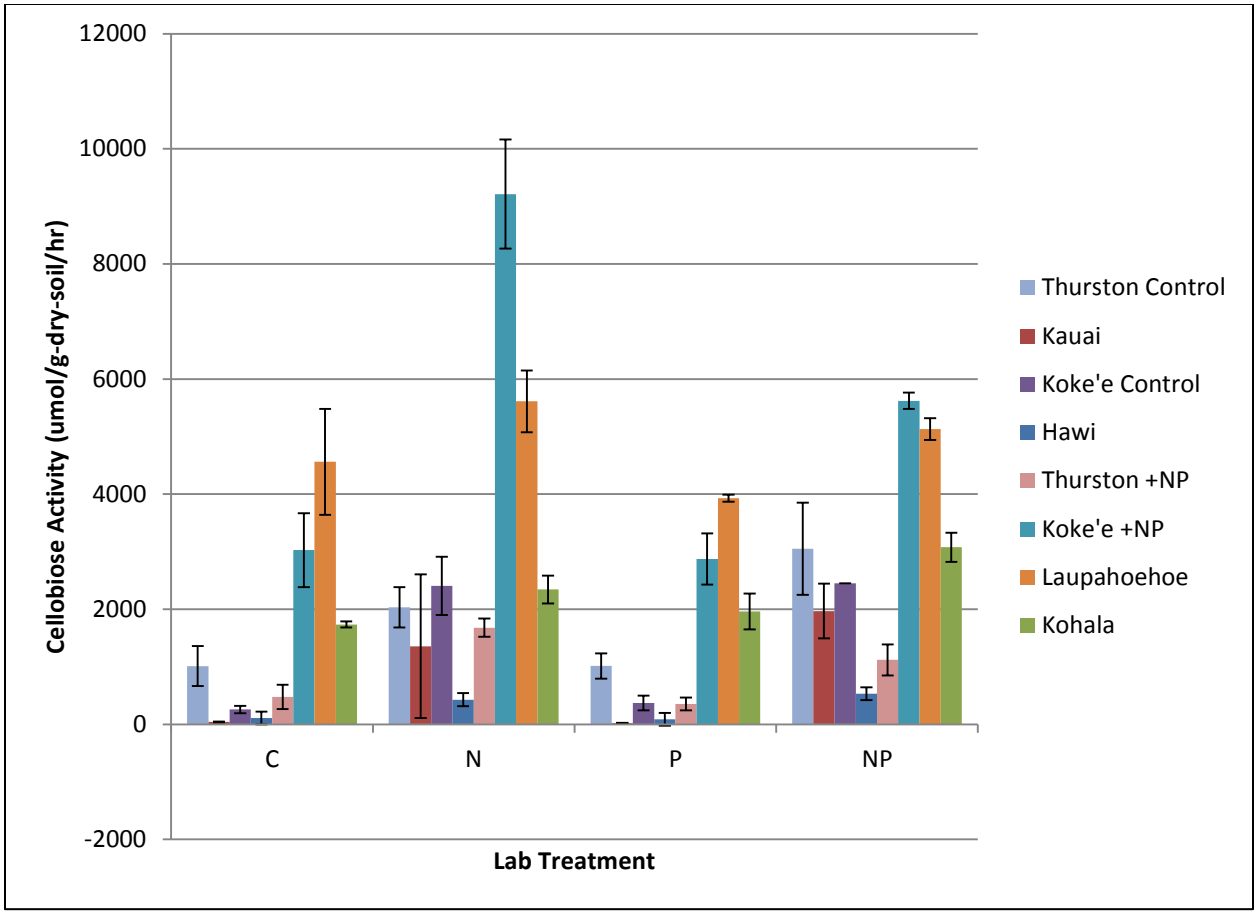


Figure 14. Response of xylosidase enzyme activity to nutrient additions.

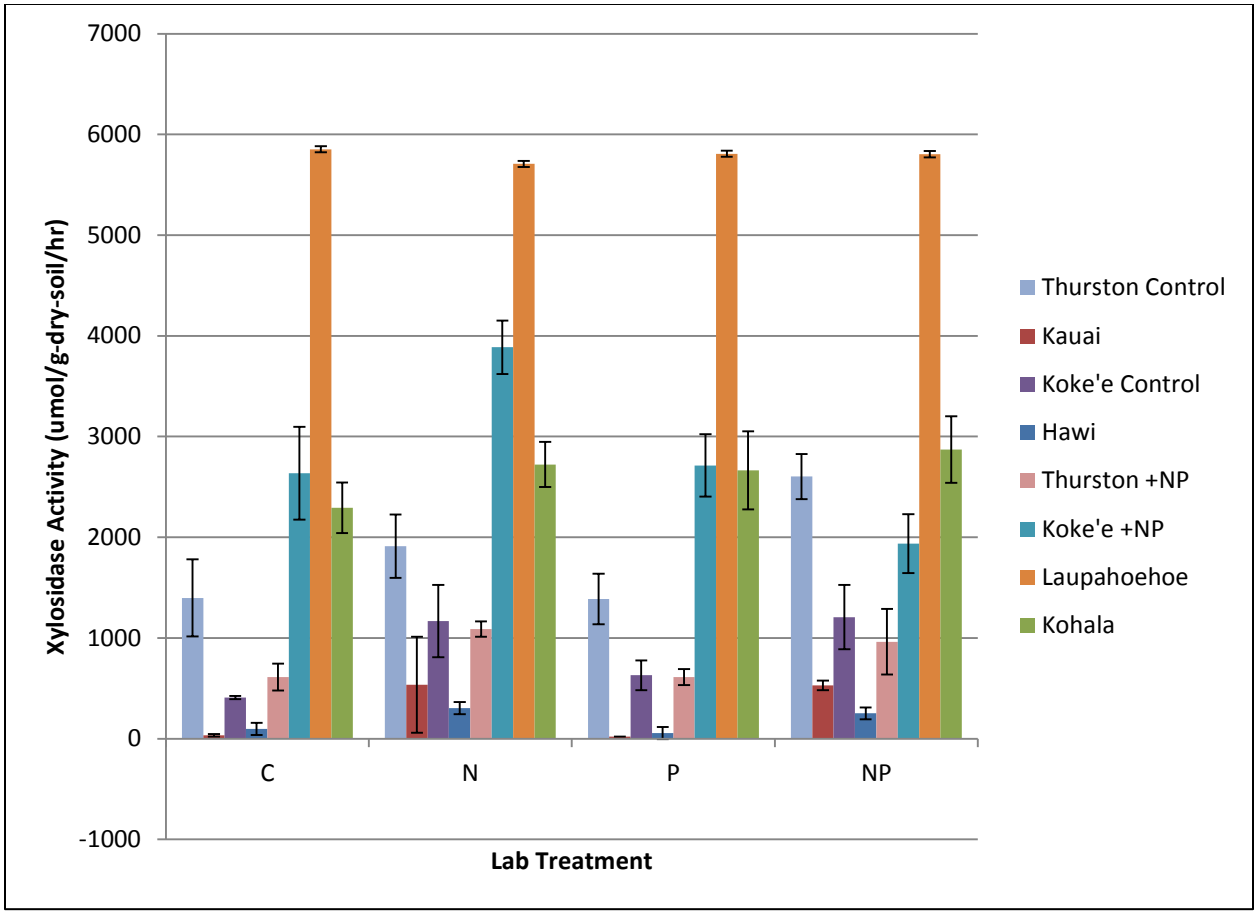


Figure 15. Response of N-Acetylglucosamine enzyme activity to nutrient additions.

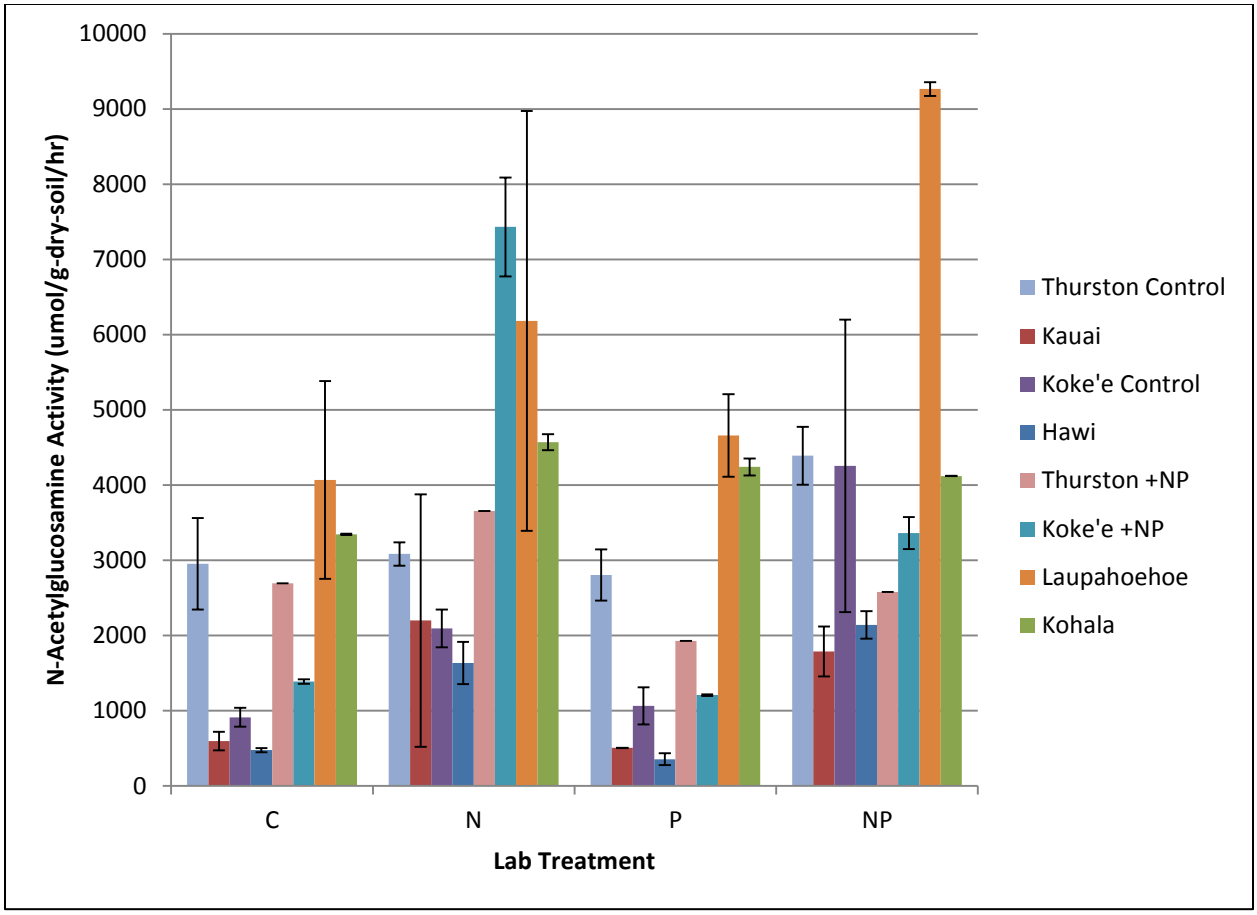


Figure 16. Response of phosphatase enzyme activity to nutrient additions.

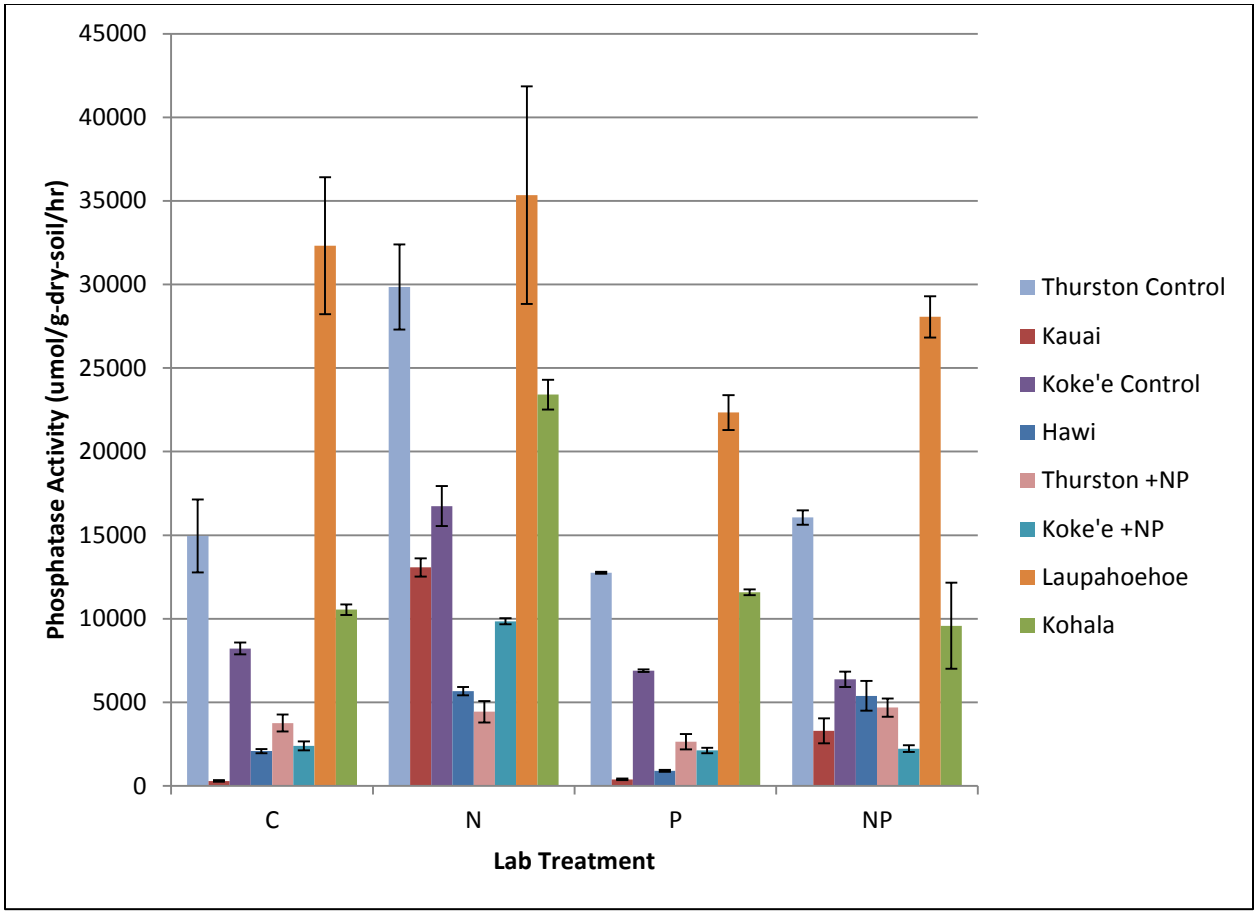
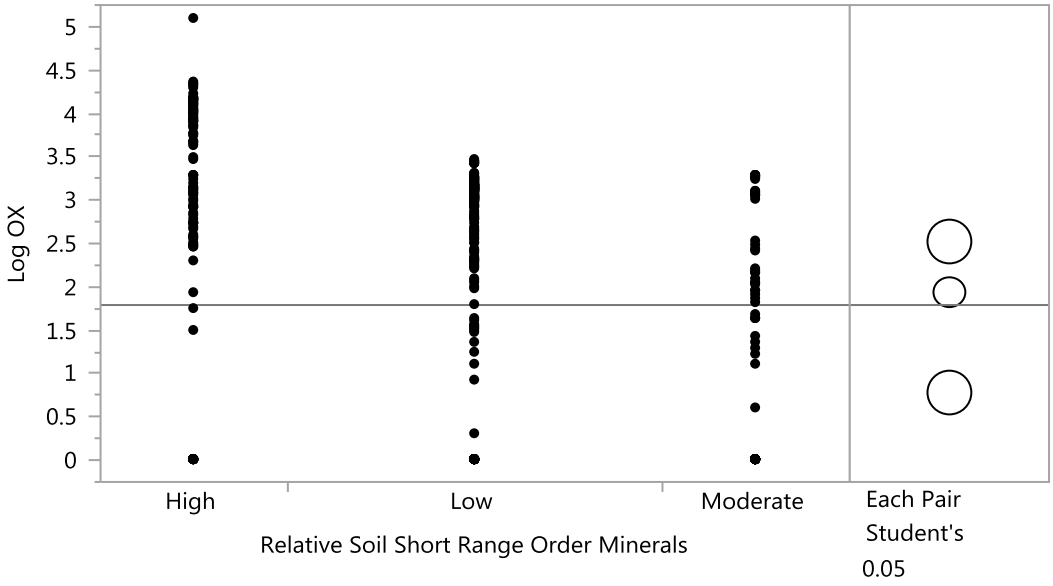


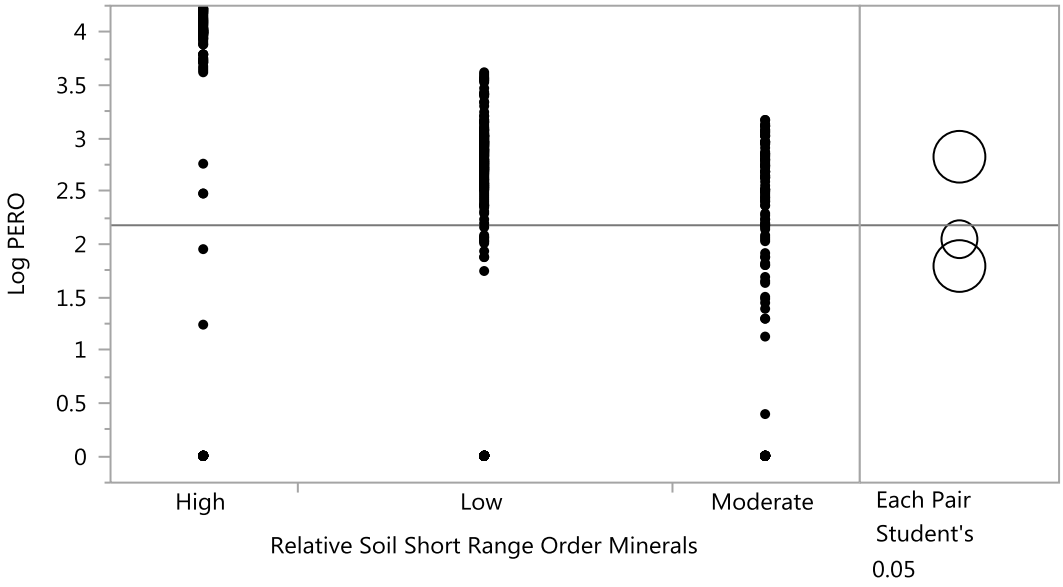
Figure 17. Differences in the nutrient addition response of the log of oxidative activity to relative soil short range order minerals (n=2).



Connecting Letters Report

Level		Mean
High	A	2.5192020
Low	B	1.9300294
Moderate	C	0.7832526

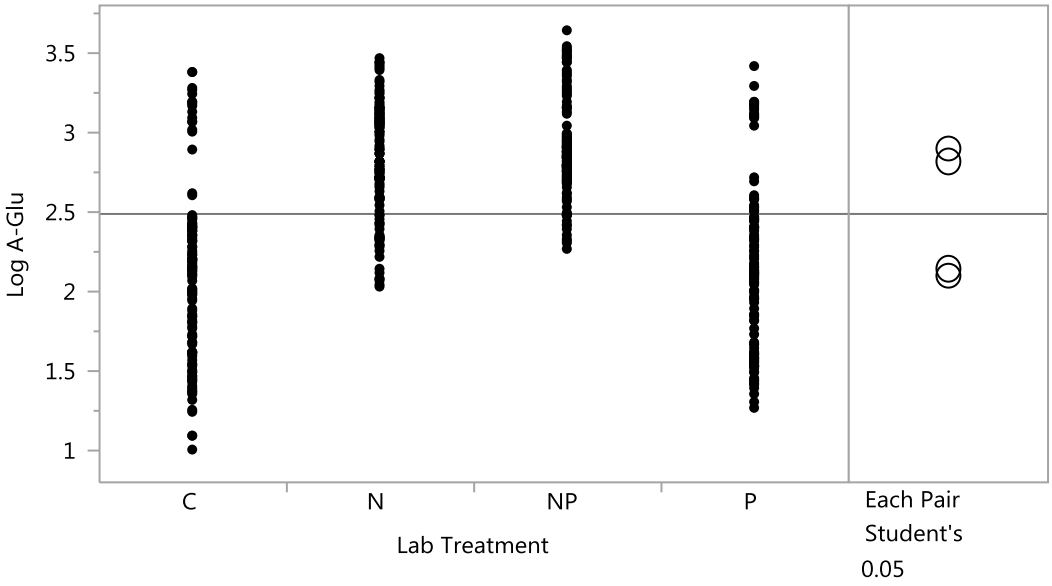
Figure 18. Differences in the nutrient addition response of the log of peroxidative activity to relative soil short range order minerals (n=2).



Connecting Letters Report

Level		Mean
High	A	2.8290085
Low	B	2.0439420
Moderate	B	1.7975414

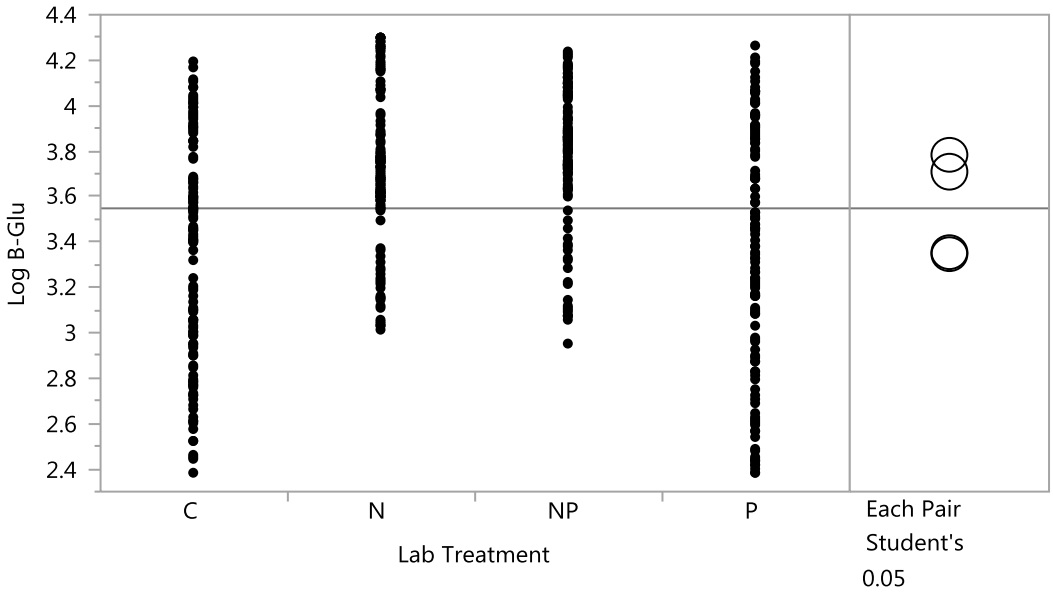
Figure 19. Differences in the nutrient addition response of alpha-glucosidase to nutrient additions (n=2).



Connecting Letters Report

Level		Mean
NP	A	2.8987655
N	A	2.8159448
P	B	2.1415607
C	B	2.1004014

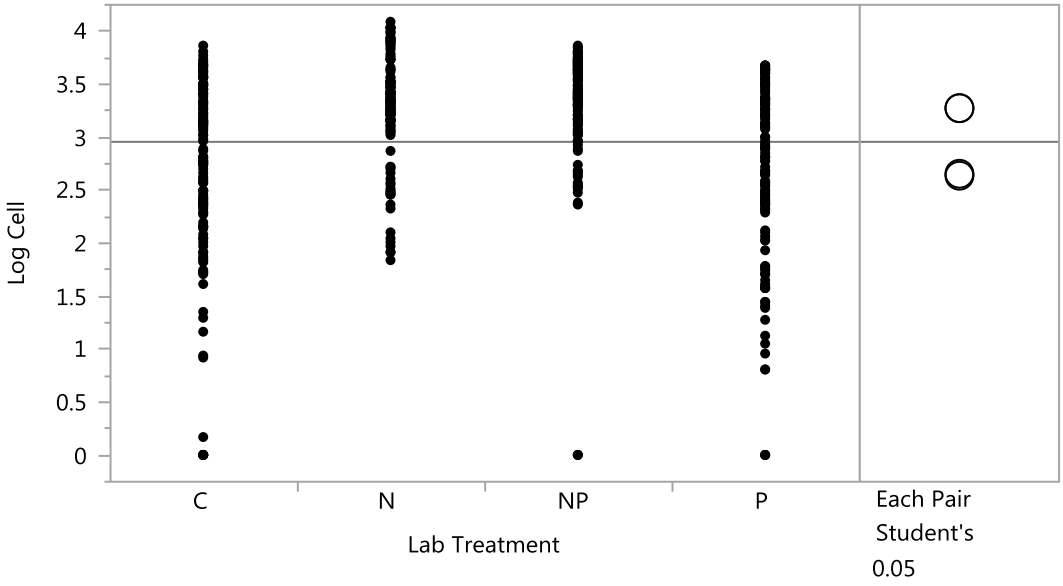
Figure 20. Differences in the nutrient addition response of beta-glucosidase to nutrient additions (n=2)**.**



Connecting Letters Report

Level		Mean
NP	A	3.7839058
N	A	3.7085014
P	B	3.3524699
C	B	3.3460337

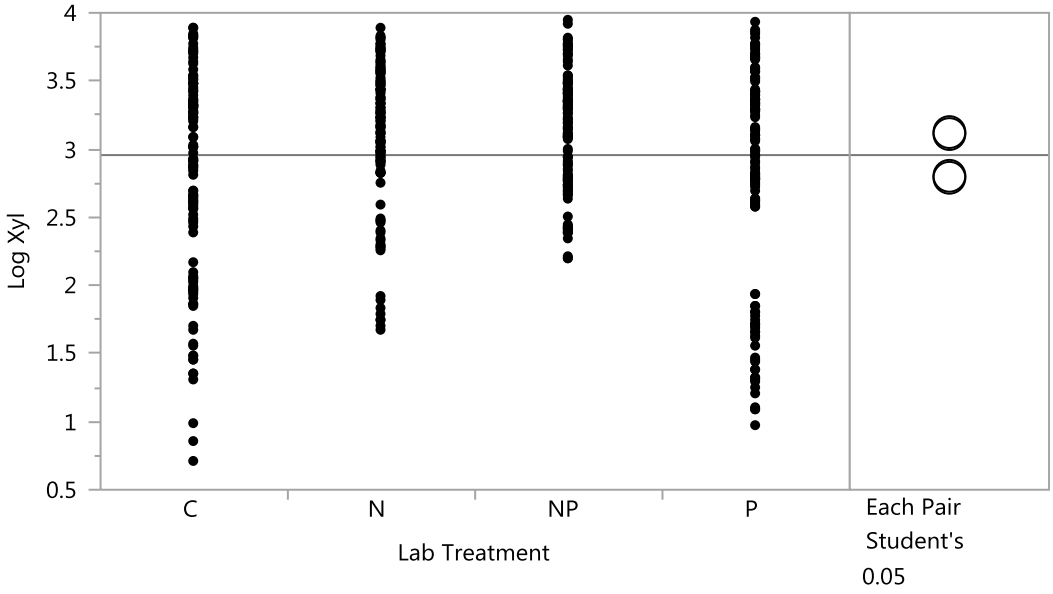
Figure 21. Differences in the nutrient addition response of cellobiose to nutrient additions (n=2)**.**



Connecting Letters Report

Level		Mean
N	A	3.2719622
NP	A	3.2688027
P	B	2.6519999
C	B	2.6387562

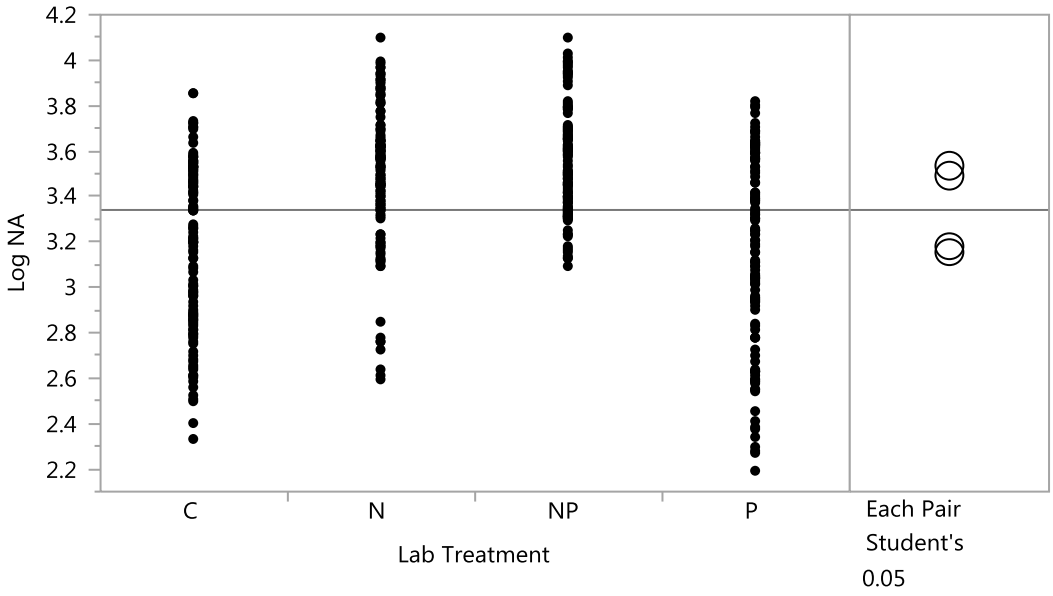
Figure 22. Differences in the nutrient addition response of xylosidase to nutrient additions (n=2)**.**



Connecting Letters Report

Level		Mean
NP	A	3.1254731
N	A	3.1109217
C	B	2.8007059
P	B	2.7879468

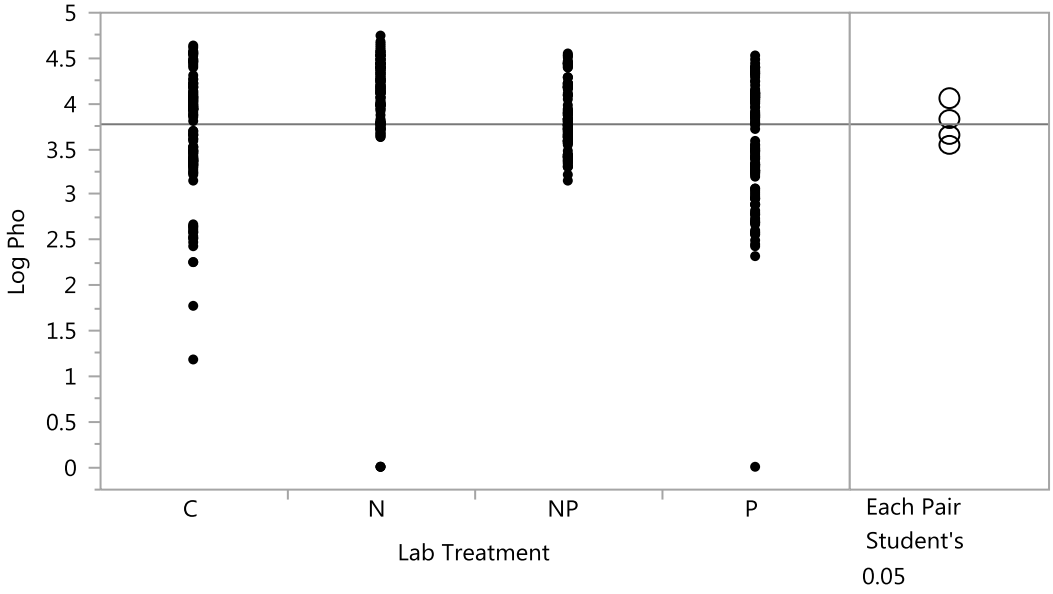
Figure 23. Differences in the nutrient addition response of N-Acetylglucosamine to nutrient additions (n=2)**.**



Connecting Letters Report

Level		Mean
NP	A	3.5348075
N	A	3.4916629
C	B	3.1802408
P	B	3.1554151

Figure 24. Differences in the nutrient addition response of phosphatase to nutrient additions (n=2)**.**



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Level		Mean
N	A	4.0605856
NP	B	3.8303729
C	C	3.6553667
P	C	3.5438331

Figure 25. Oxidative enzyme activity response at 4 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

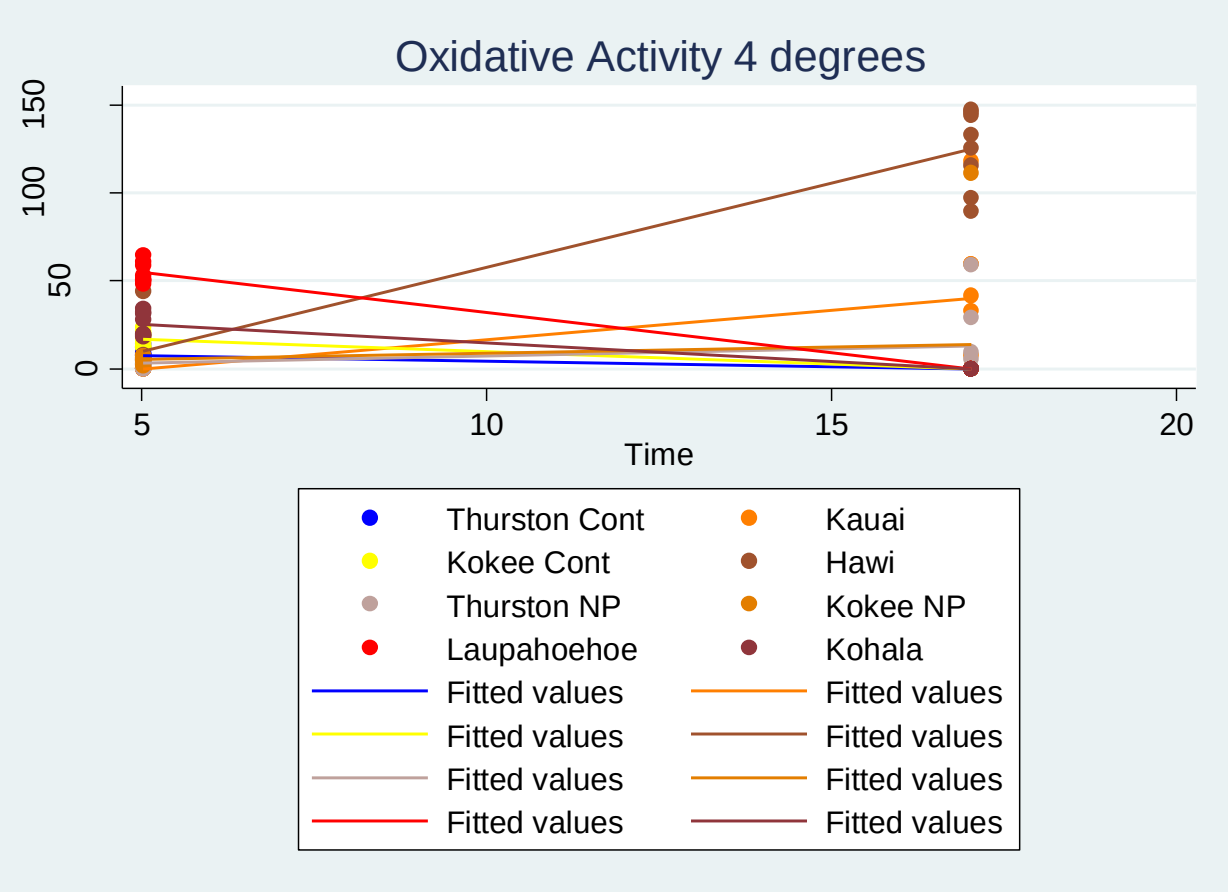


Figure 26. Oxidative enzyme activity response at 10 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

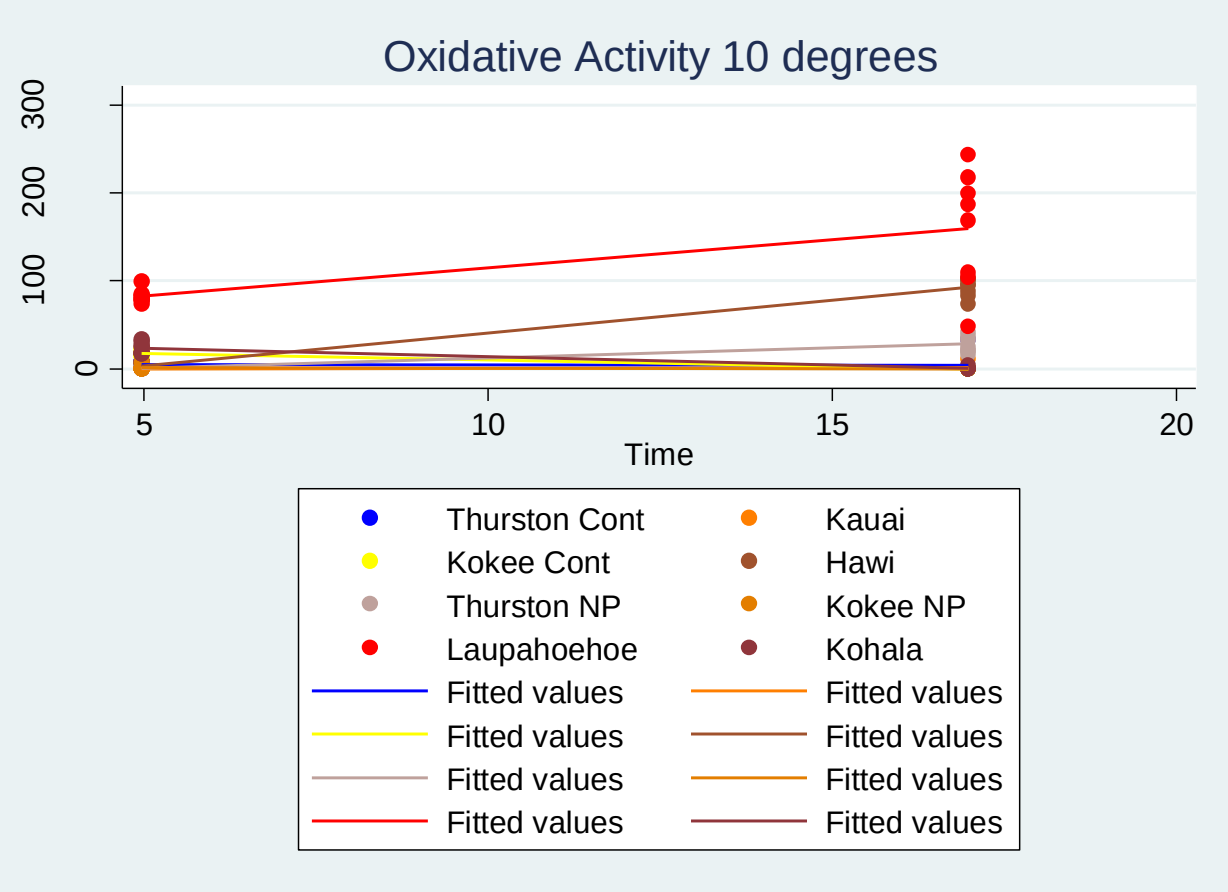


Figure 27. Oxidative enzyme activity response at 16 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

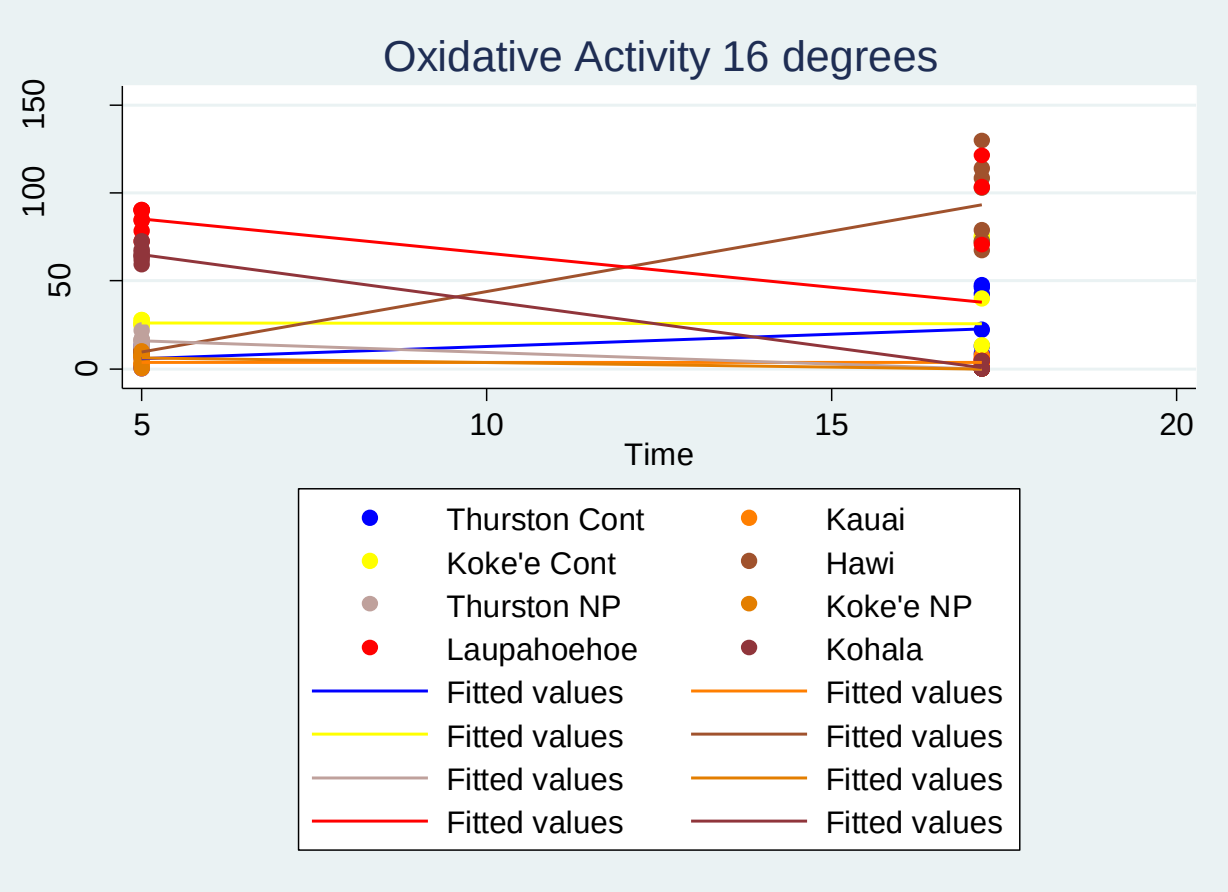


Figure 28. Oxidative enzyme activity response at 22 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

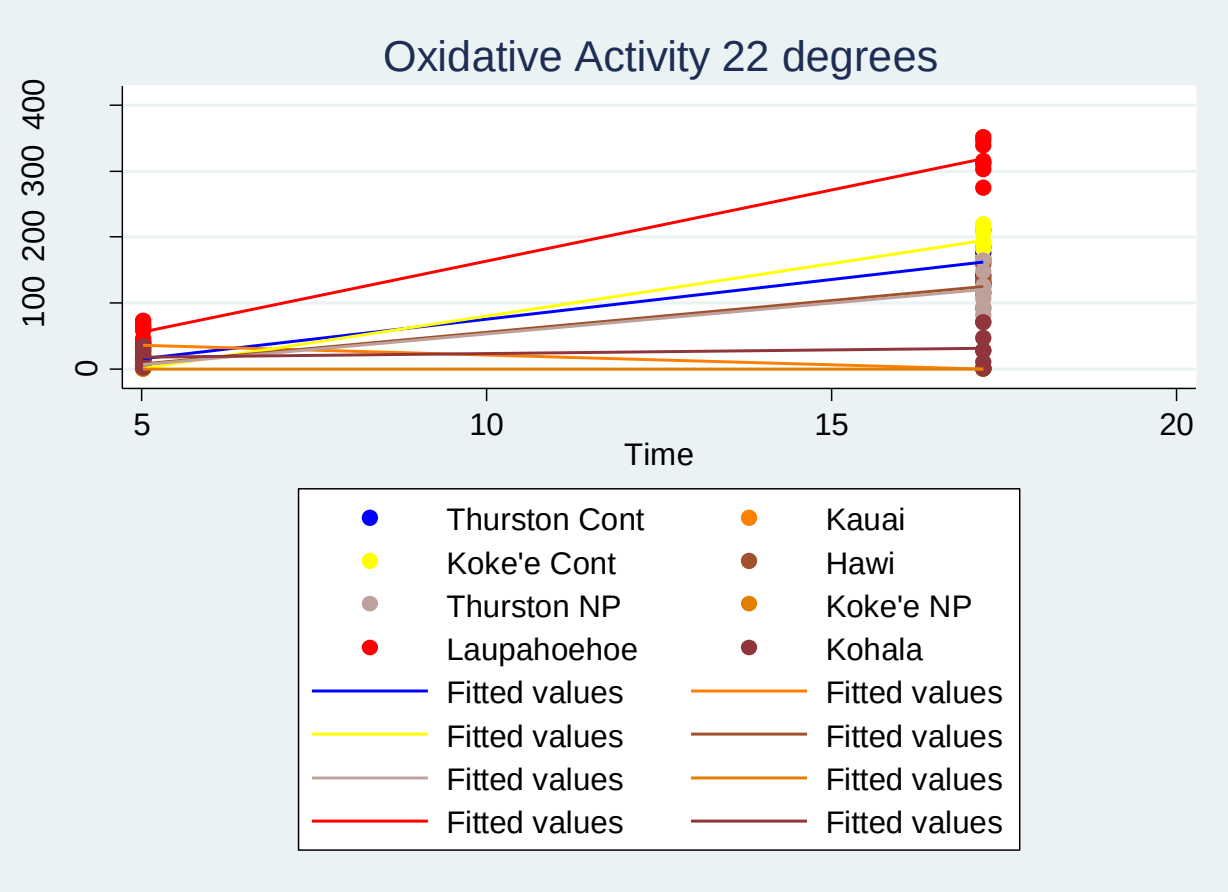


Figure 29. Oxidative enzyme activity response at 27 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

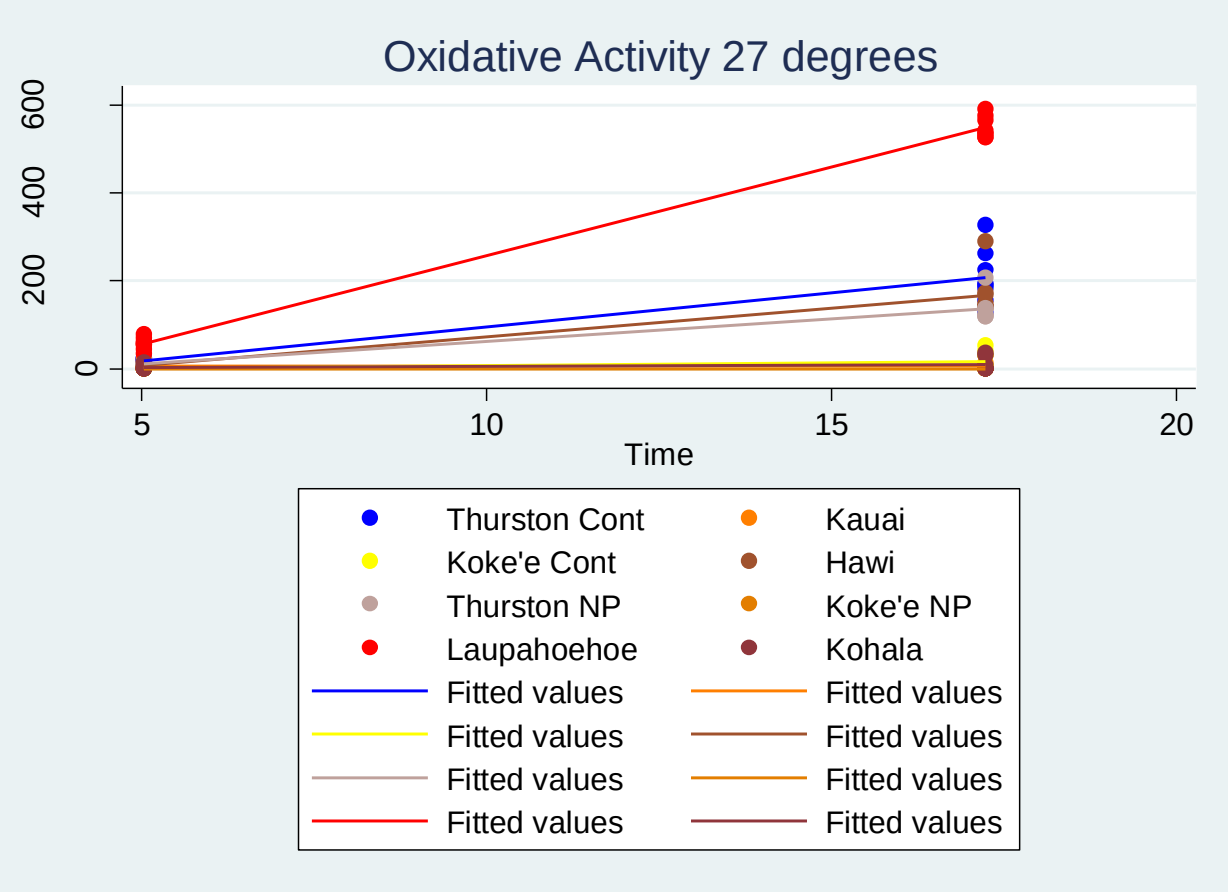


Figure 30. Oxidative enzyme activity response at 34 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

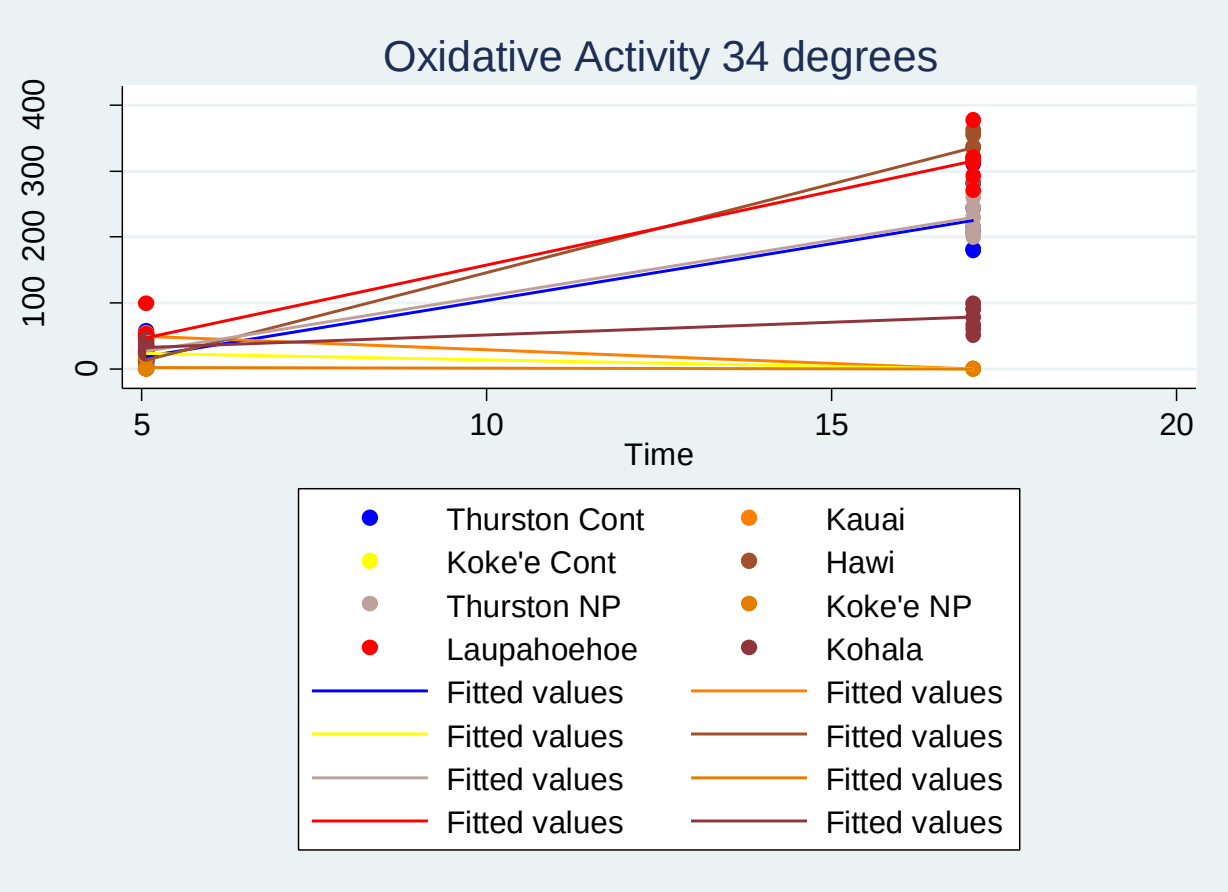


Figure 31. Oxidative enzyme activity response at 40 degrees Celsius over two time points (~ 5 hours and ~ 17 hours).

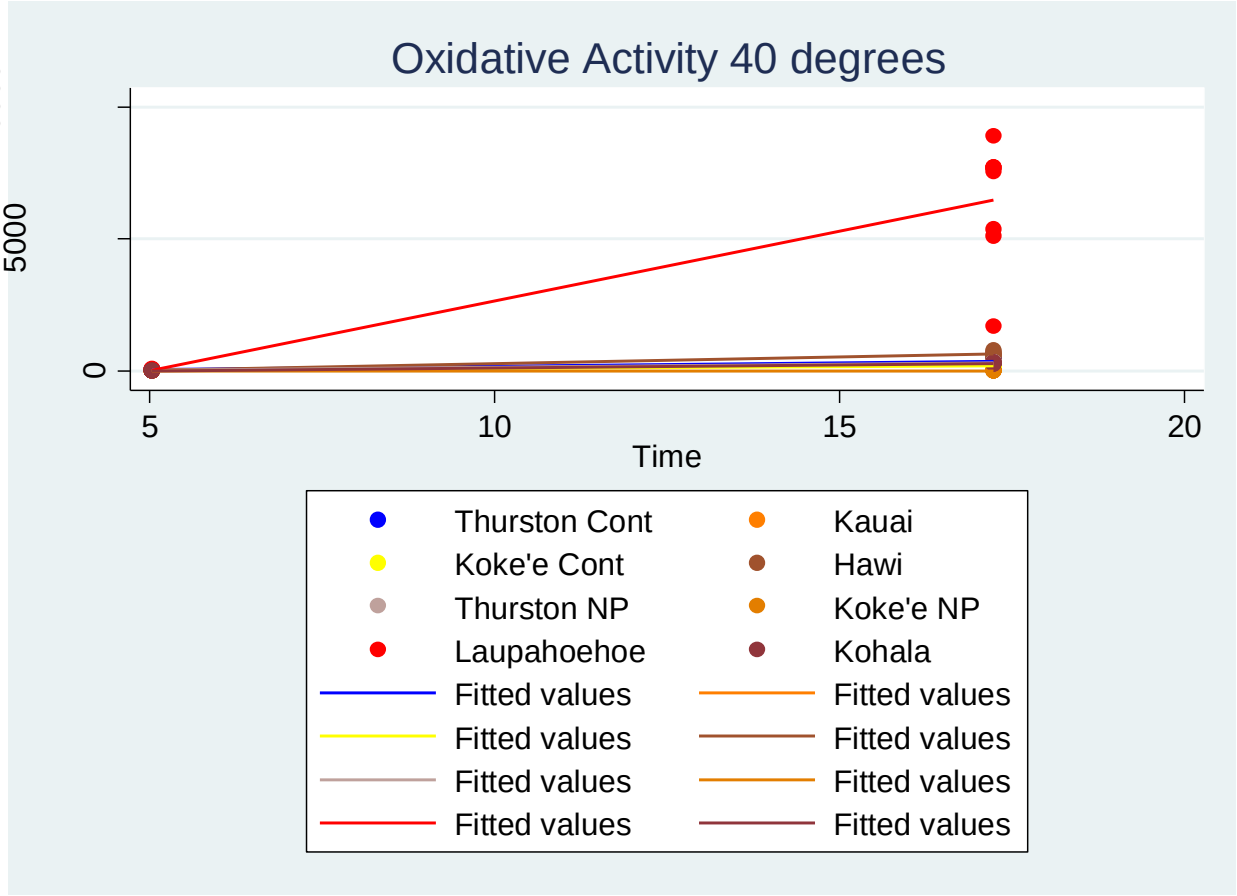


Figure 32. Response of log oxidative enzyme activity to changes in temperature.

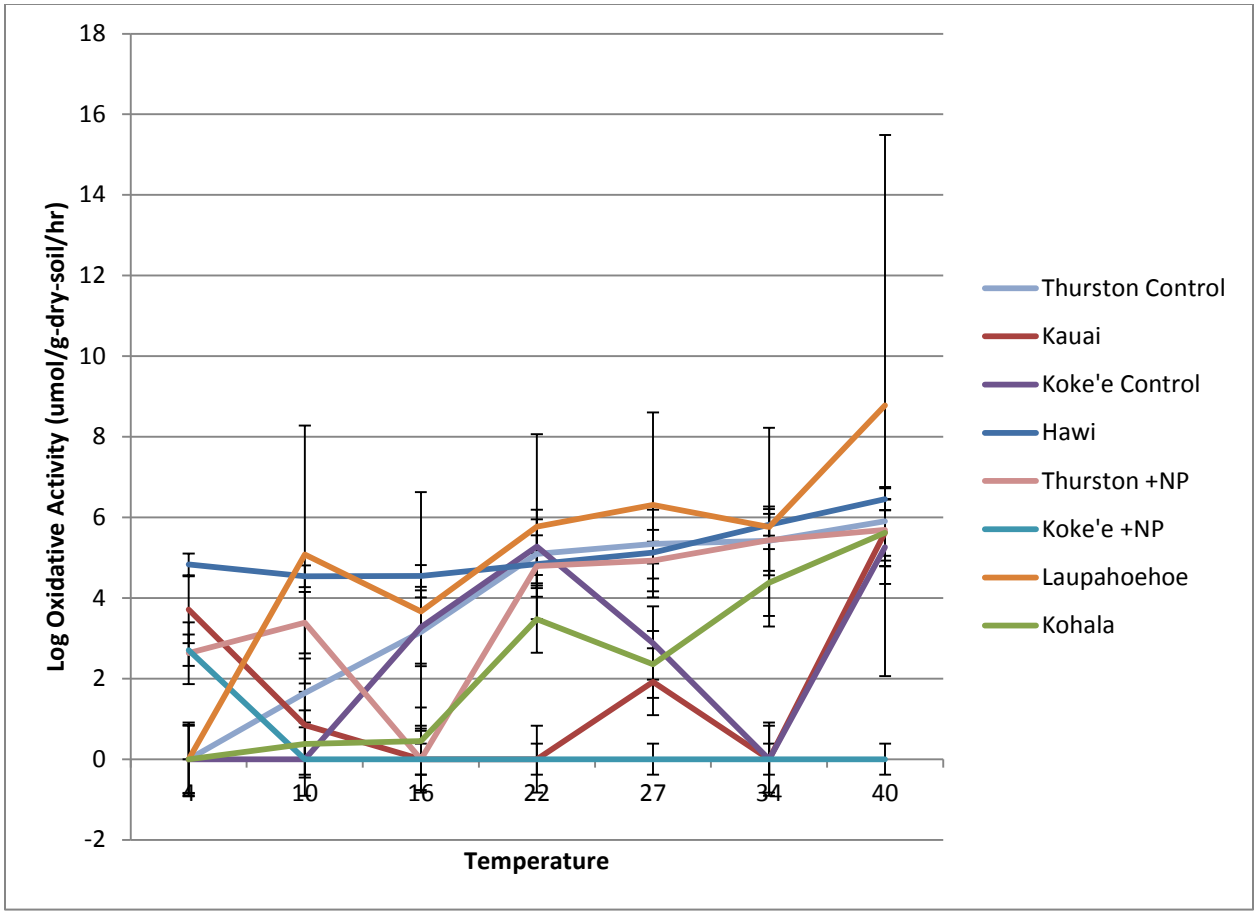


Figure 33. Response of peroxidative enzyme activity to changes in temperature.

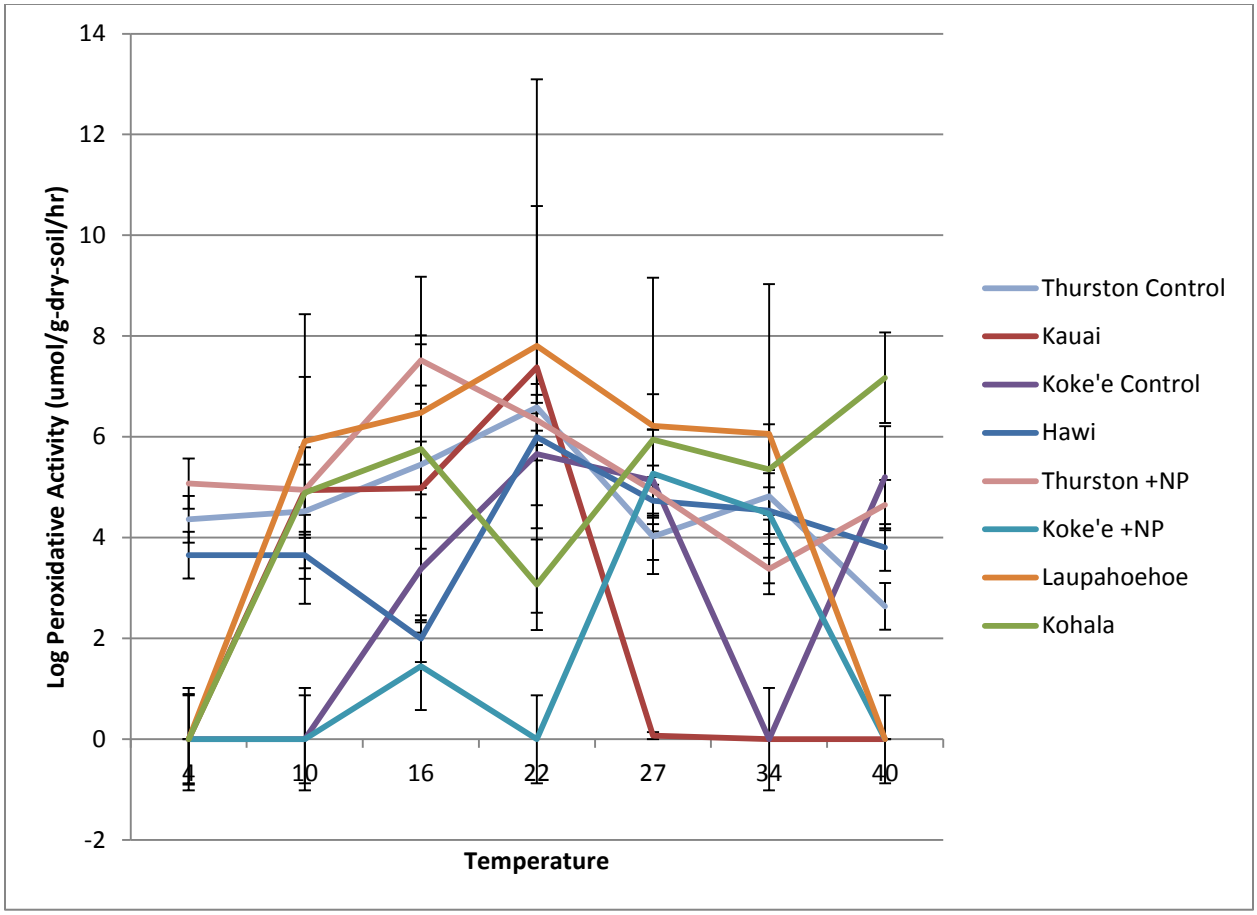


Figure 34. Response of alpha-glucosidase enzyme activity to changes in temperature.

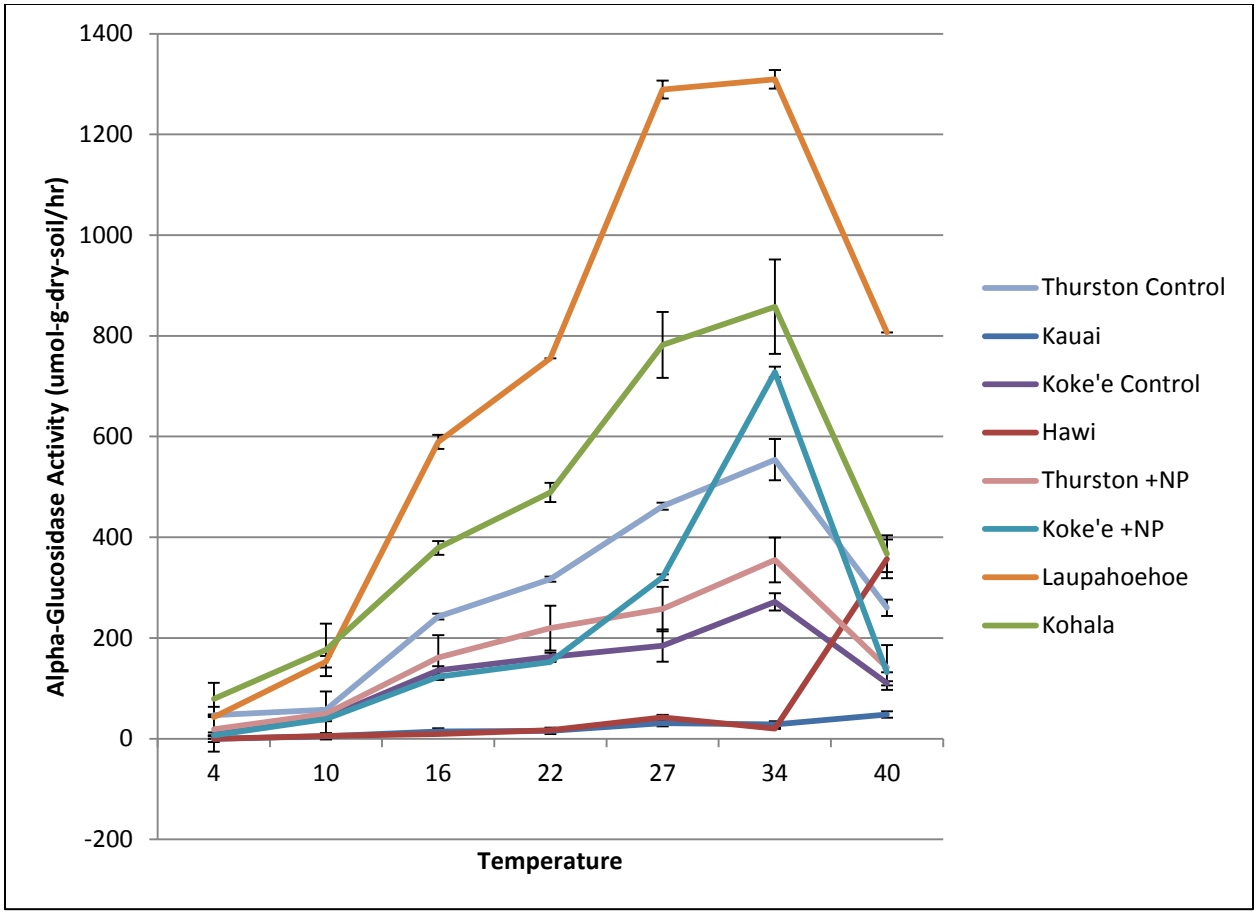


Figure 35. Response of beta-glucosidase enzyme activity to changes in temperature.

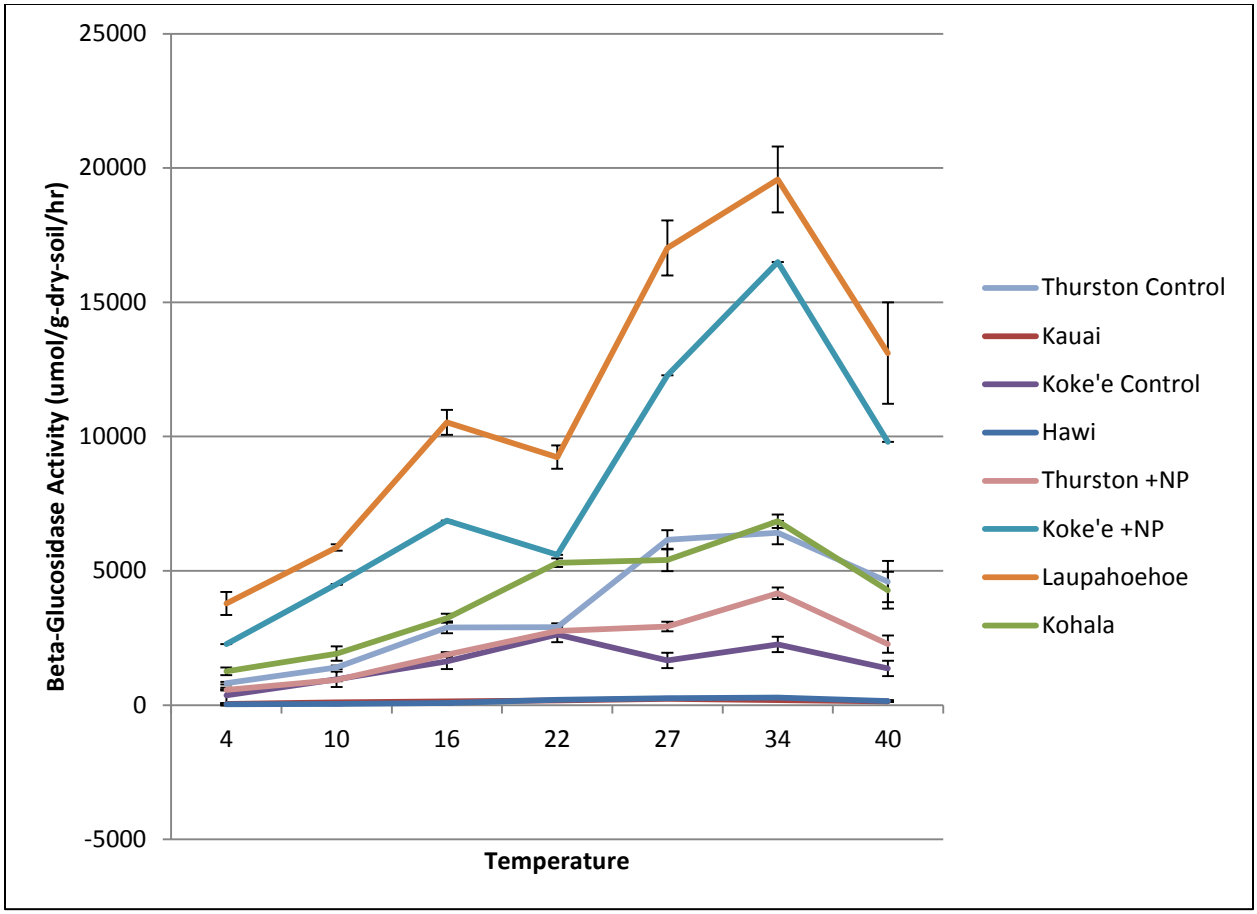


Figure 36. Response of cellobiose enzyme activity to changes in temperature.

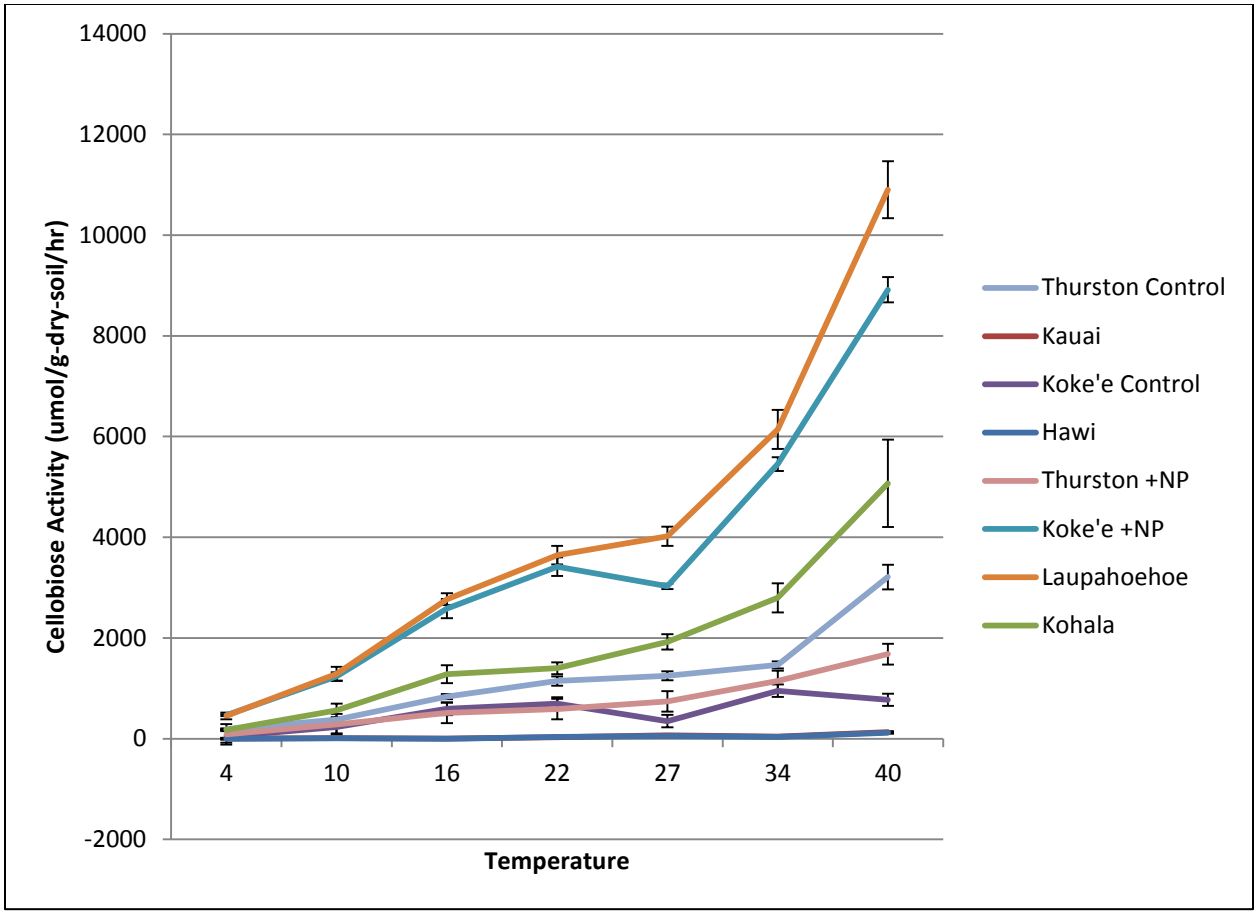


Figure 37. Response of xylosidase enzyme activity to changes in temperature.

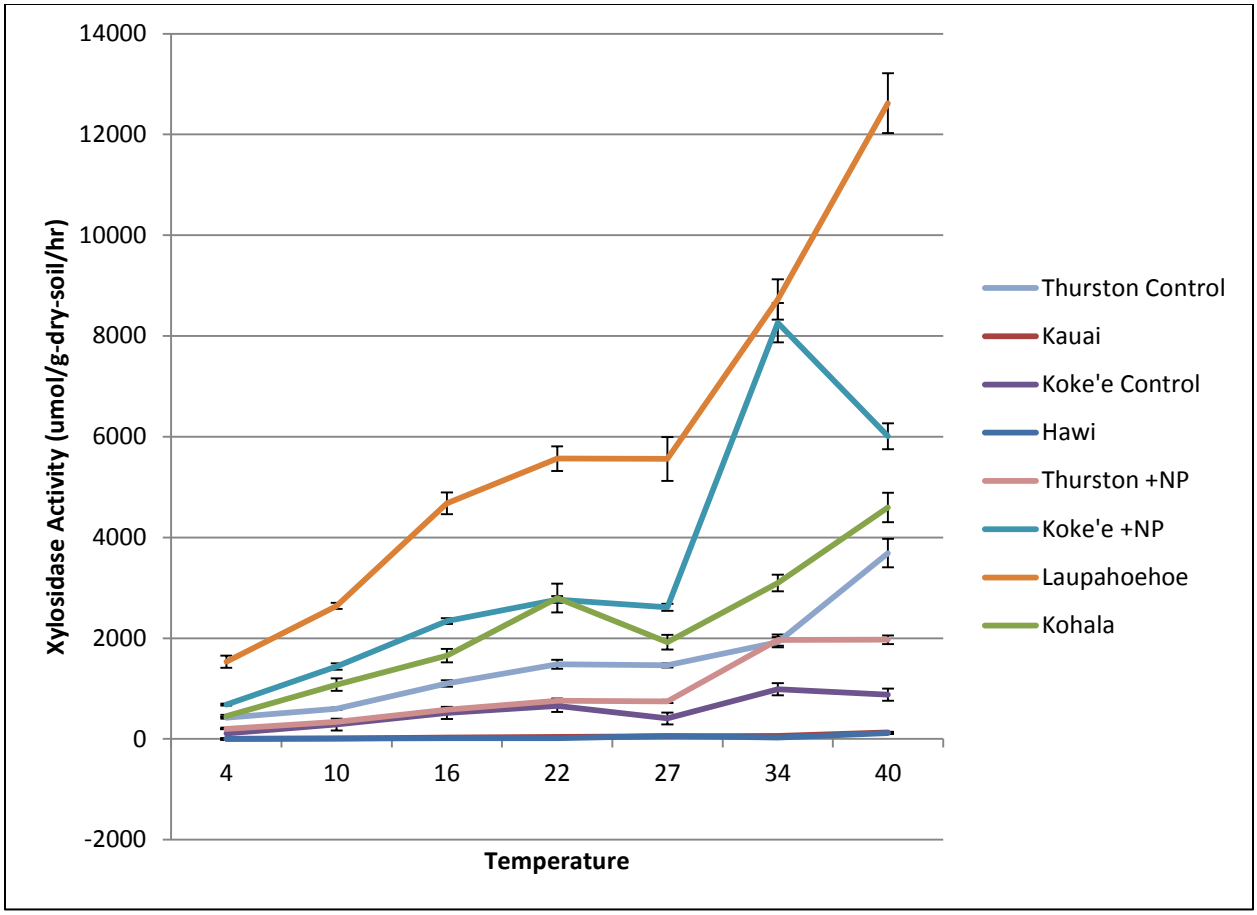


Figure 38. Response of N-Acetylglucosamine enzyme activity to changes in temperature.

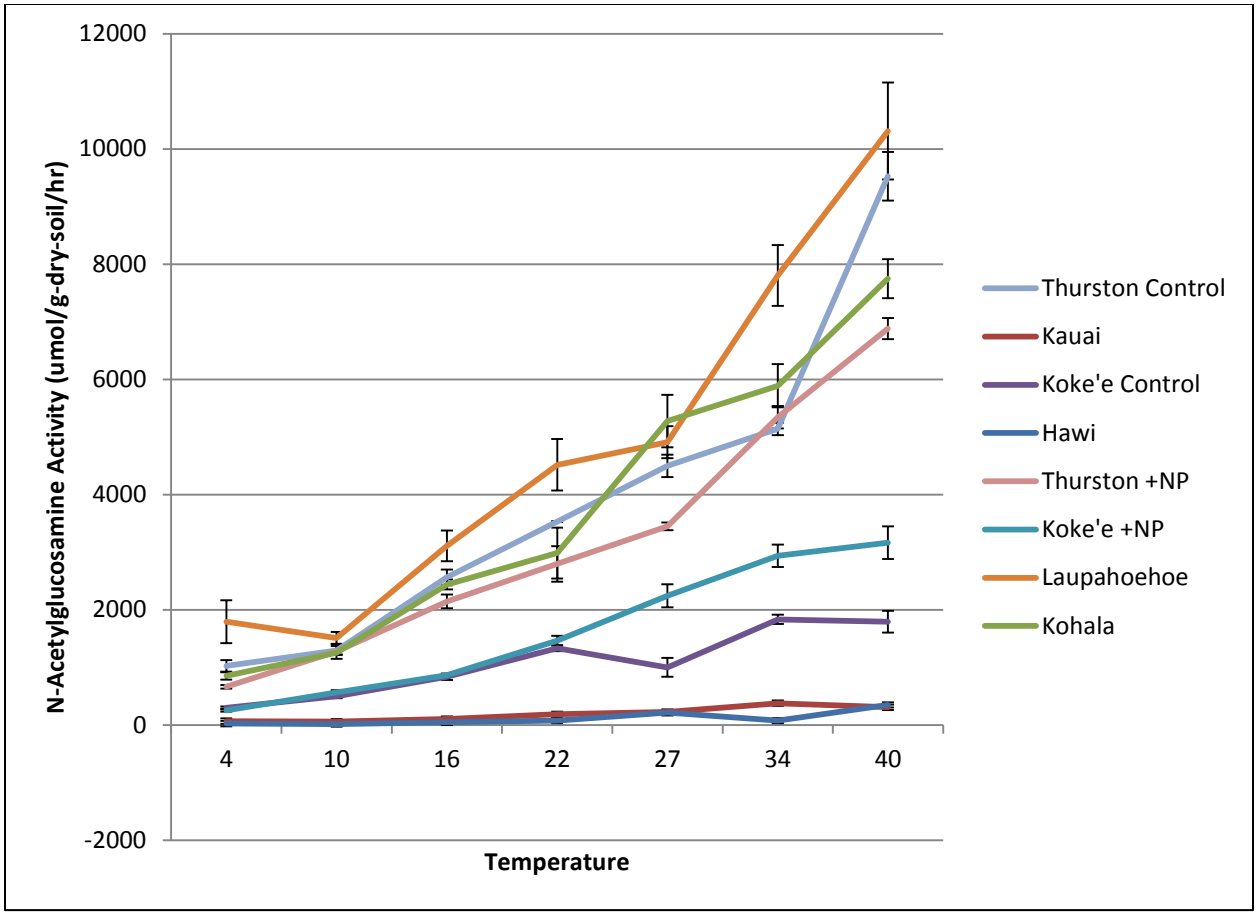


Figure 39. Response of phosphatase enzyme activity to changes in temperature.

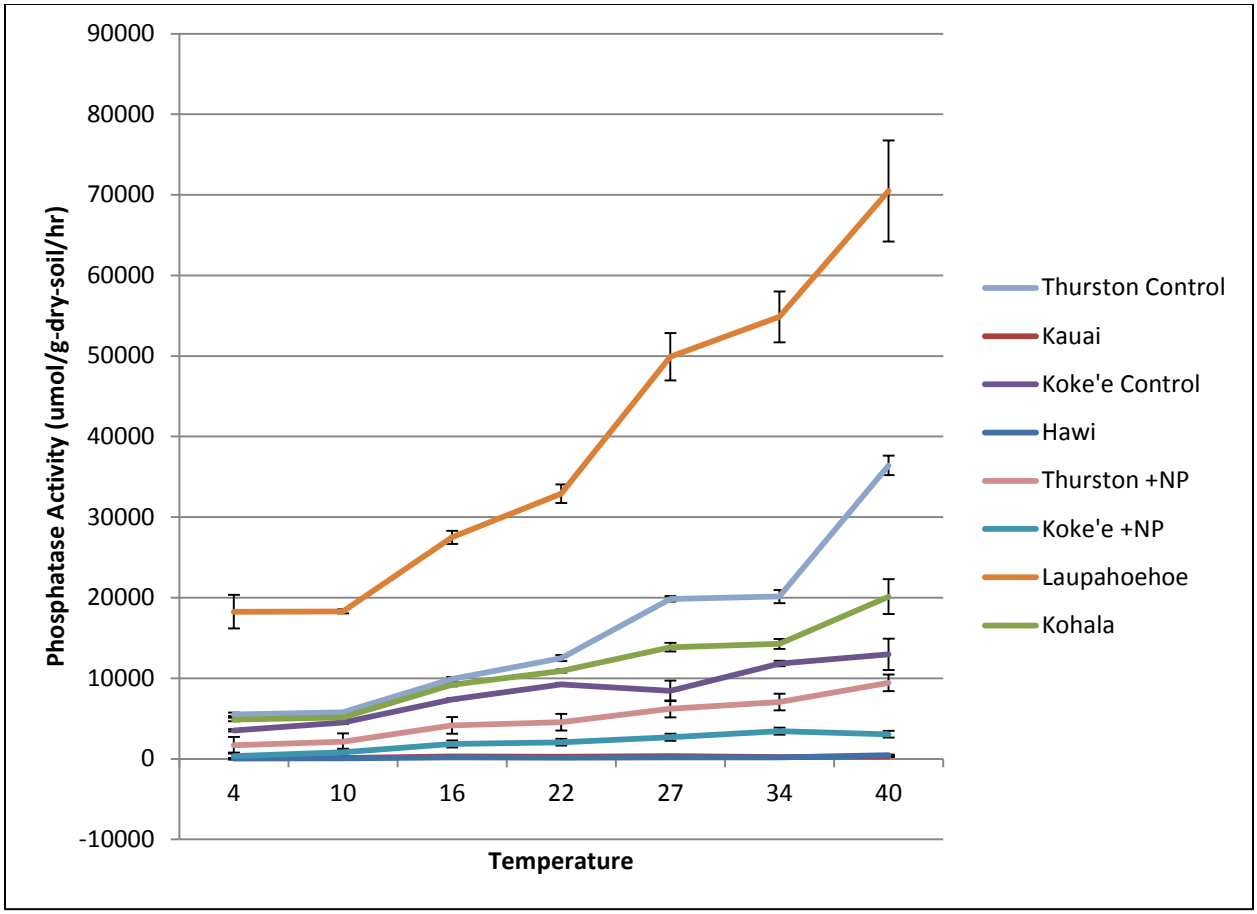
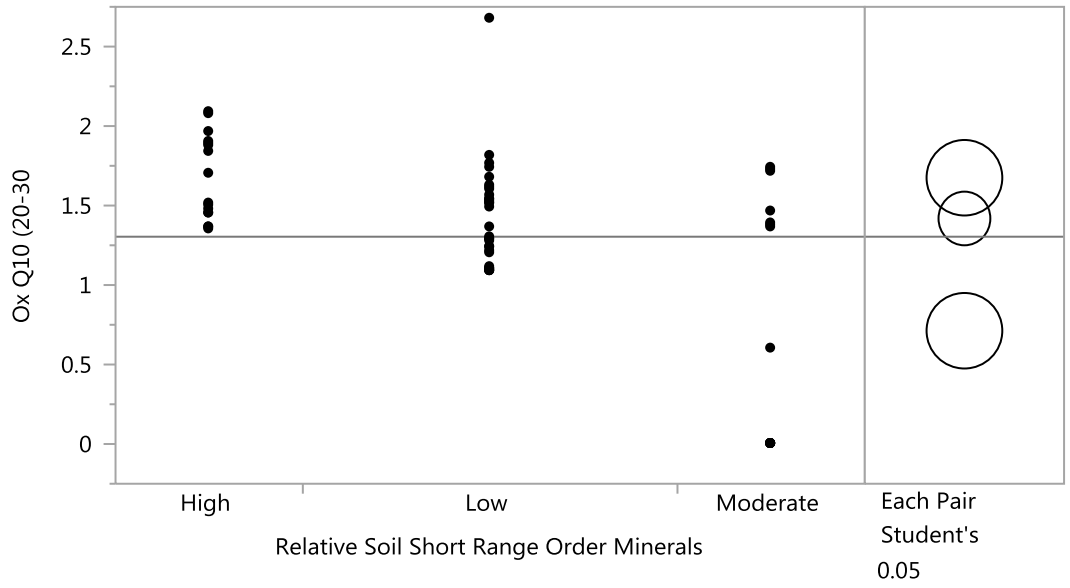


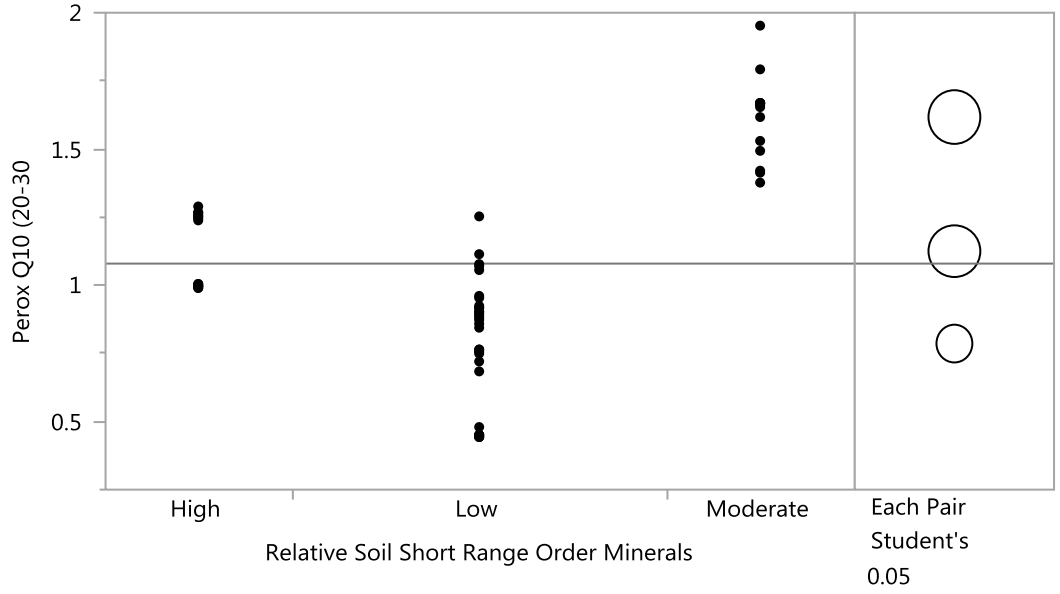
Figure 40. Differences in the oxidative Q_{10} response to relative short range order minerals (n=8).



Connecting Letters Report

Level		Mean
High	A	1.6735634
Low	A	1.4161516
Moderate	B	0.7098805

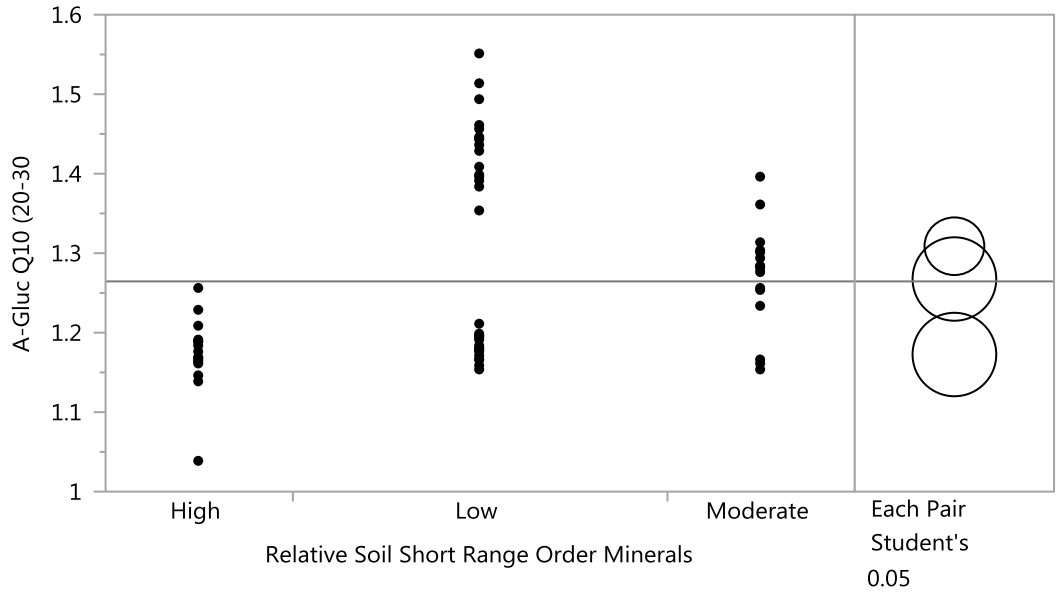
Figure 41. Differences in the peroxidative Q_{10} response to relative short range order minerals (n=8).



Connecting Letters Report

Level				Mean
Moderate	A			1.6169921
High		B		1.1239123
Low			C	0.7883053

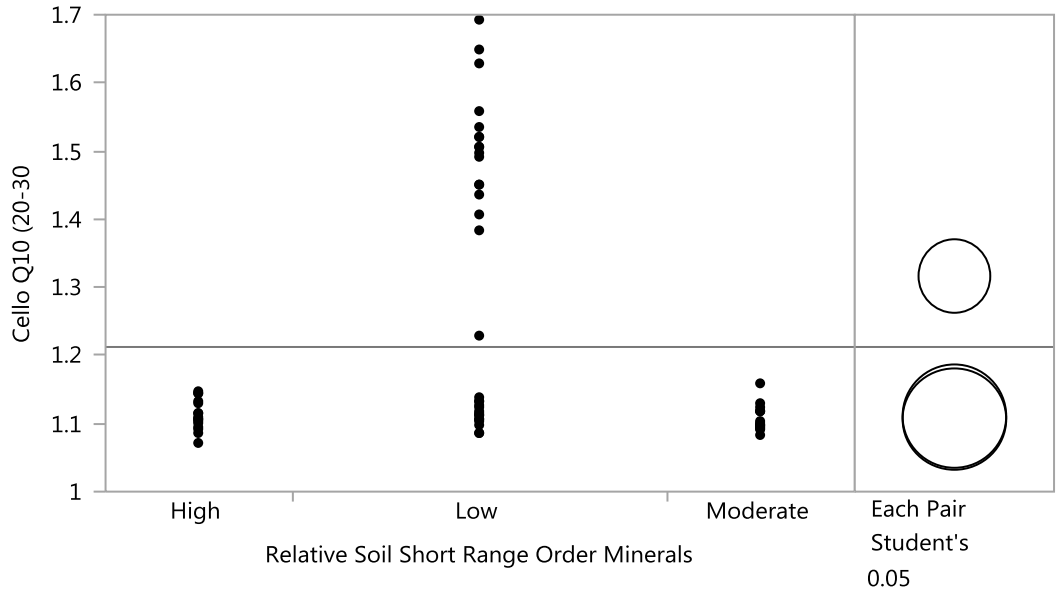
Figure 42. Differences in the alpha-glucosidase Q_{10} response to relative short range order minerals (n=8).



Connecting Letters Report

Level			Mean
Low	A		1.3086021
Moderate	A		1.2685899
High		B	1.1721995

Figure 43. Differences in the cellobiose Q_{10} response to relative short range order minerals (n=8).



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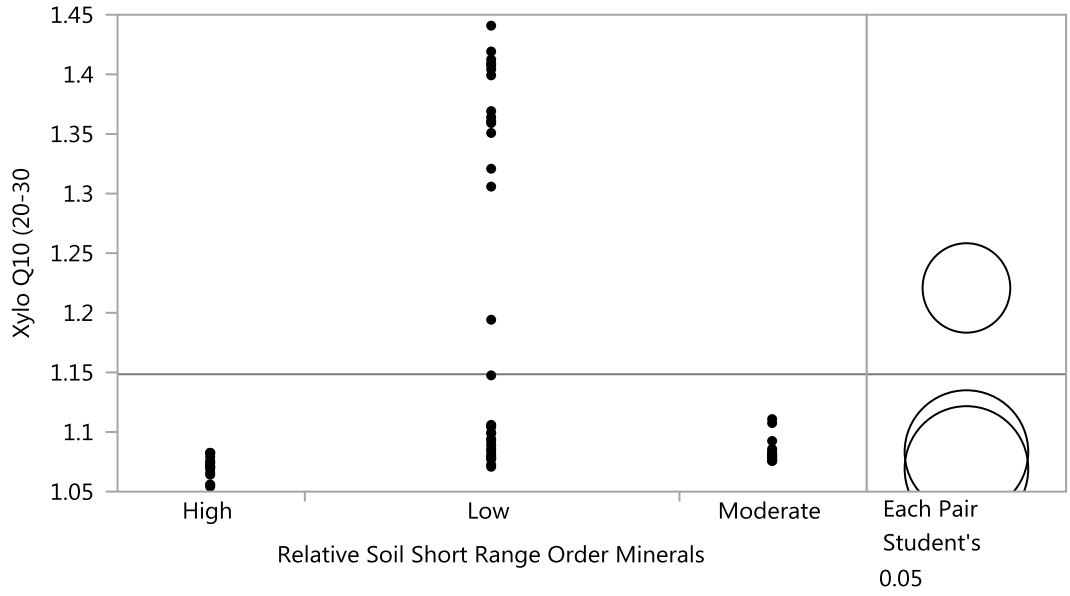
Level		Mean
Low	A	1.3159786
High	B	1.1108181
Moderate	B	1.1064910

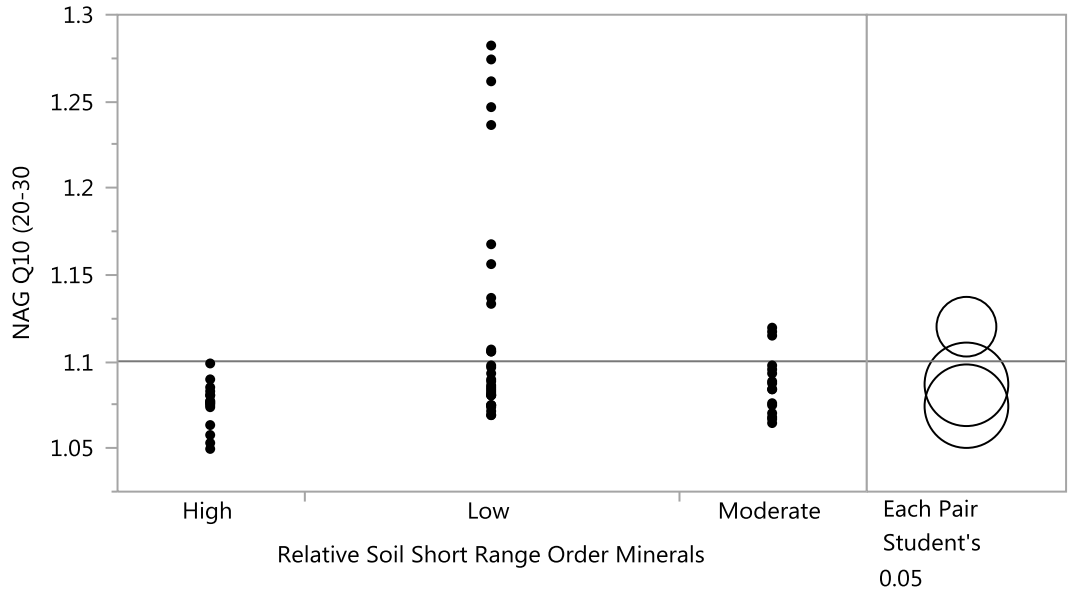
Figure 44. Differences in the xylosidase Q_{10} response to relative short range order minerals (n=8).



Connecting Letters Report

Level		Mean
Low	A	1.2205571
Moderate	B	1.0831465
High	B	1.0695197

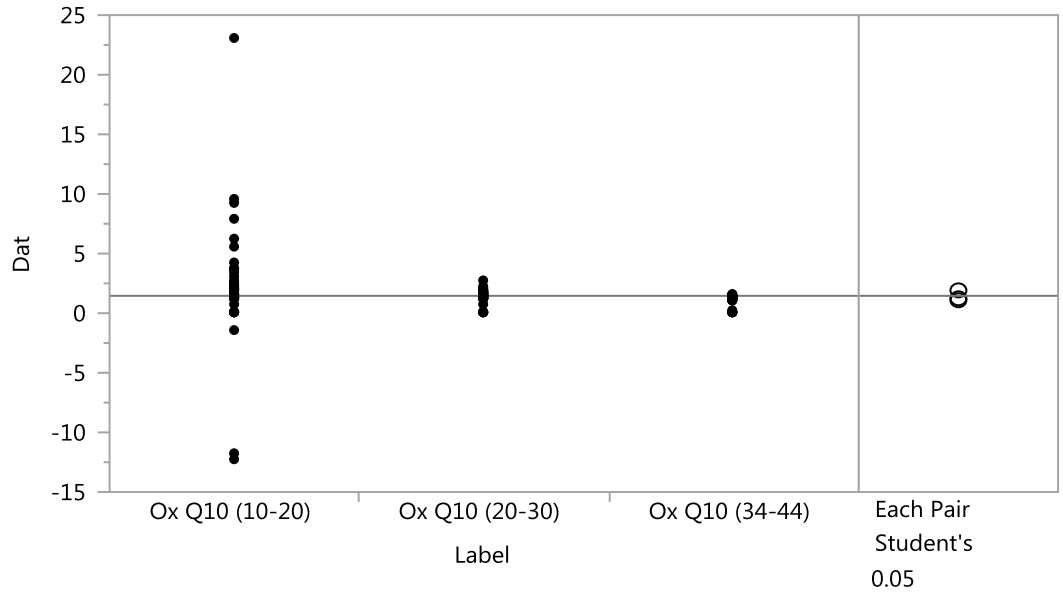
Figure 45. Differences in the n-acetylglucosamine Q_{10} response to relative short range order minerals (n=8).



Connecting Letters Report

Level			Mean
Low	A		1.1196896
Moderate		B	1.0871984
High		B	1.0740647

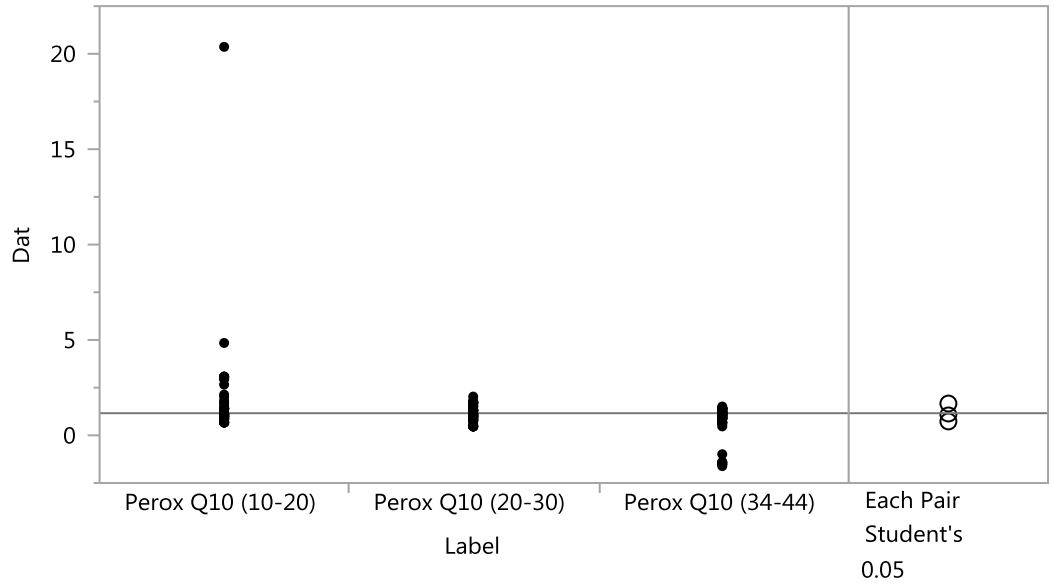
Figure 46. Temperature kinetics oxidative response for three Q_{10} groups.



Connecting Letters Report

Level			Mean
Ox Q10 (10-20)	A		1.9699469
Ox Q10 (20-30)	A	B	1.3039368
Ox Q10 (34-44)		B	1.0937047

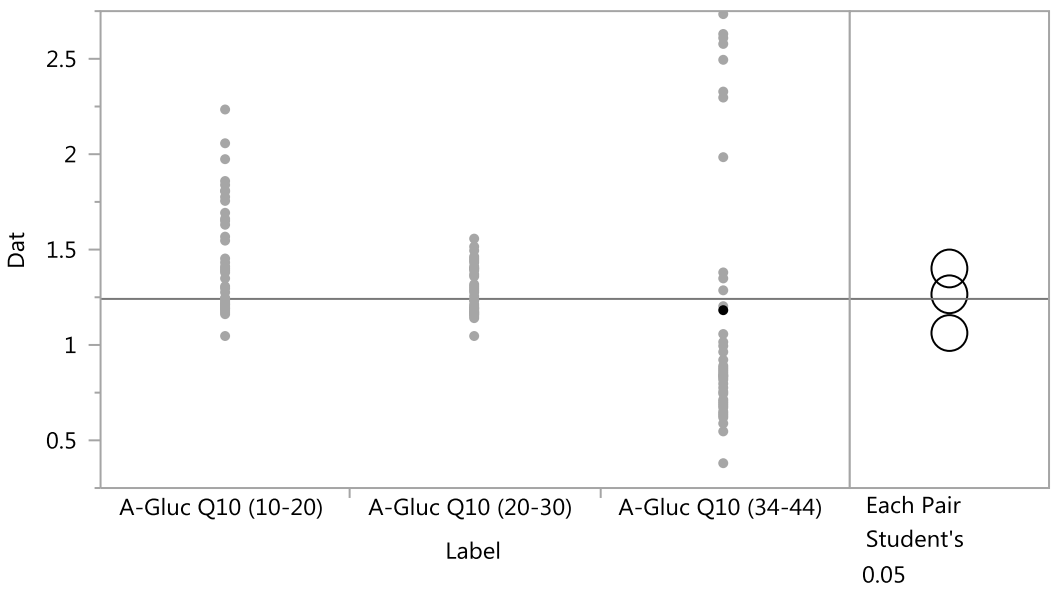
Figure 47. Temperature kinetics peroxidative response for three Q_{10} groups.



Connecting Letters Report

Level			Mean
Perox Q10 (10-20)	A		1.6639532
Perox Q10 (20-30)		B	1.0793787
Perox Q10 (34-44)		B	0.7393882

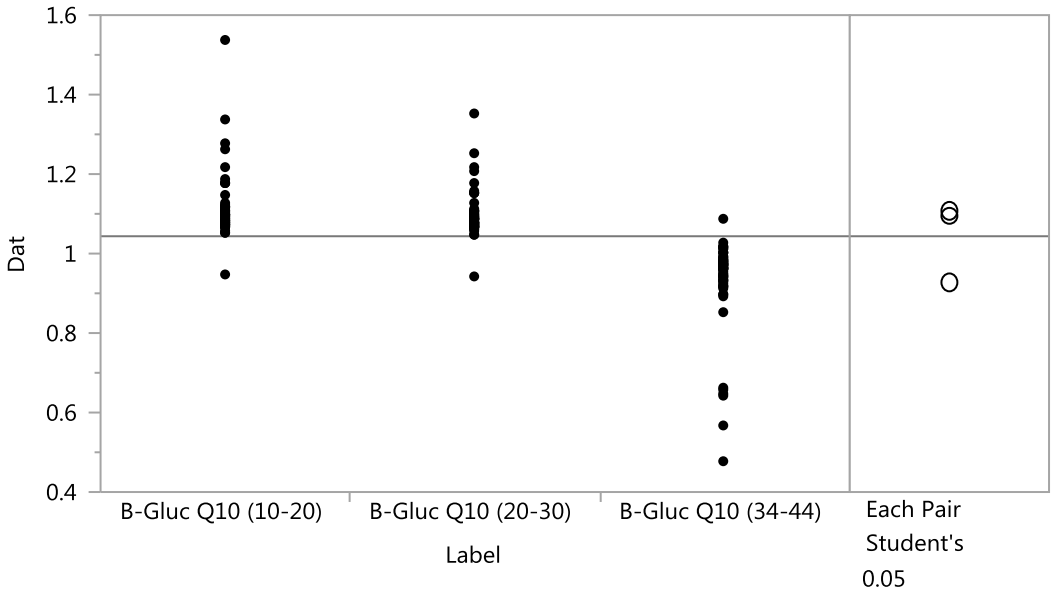
Figure 48. Temperature kinetics alpha-glucosidase response for three Q_{10} groups.



Connecting Letters Report

Level				Mean
A-Gluc Q10 (10-20)	A			1.4000656
A-Gluc Q10 (20-30)		B		1.2644984
A-Gluc Q10 (34-44)			C	1.0595647

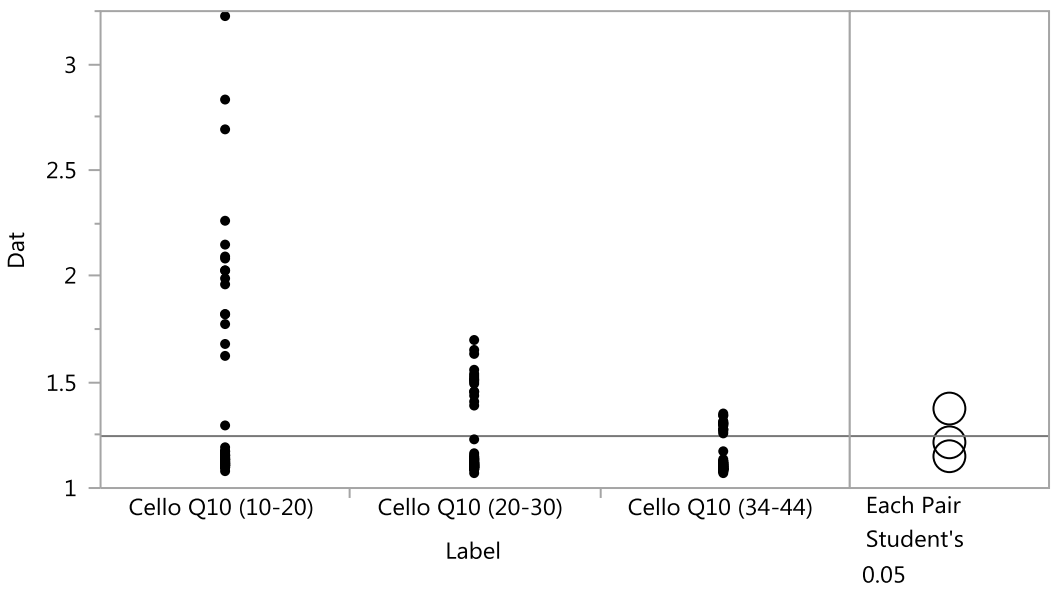
Figure 49. Temperature kinetics beta-glucosidase response for three Q_{10} groups.



Connecting Letters Report

Level			Mean
B-Gluc Q10 (10-20)	A		1.1083253
B-Gluc Q10 (20-30)	A		1.0939938
B-Gluc Q10 (34-44)		B	0.9284191

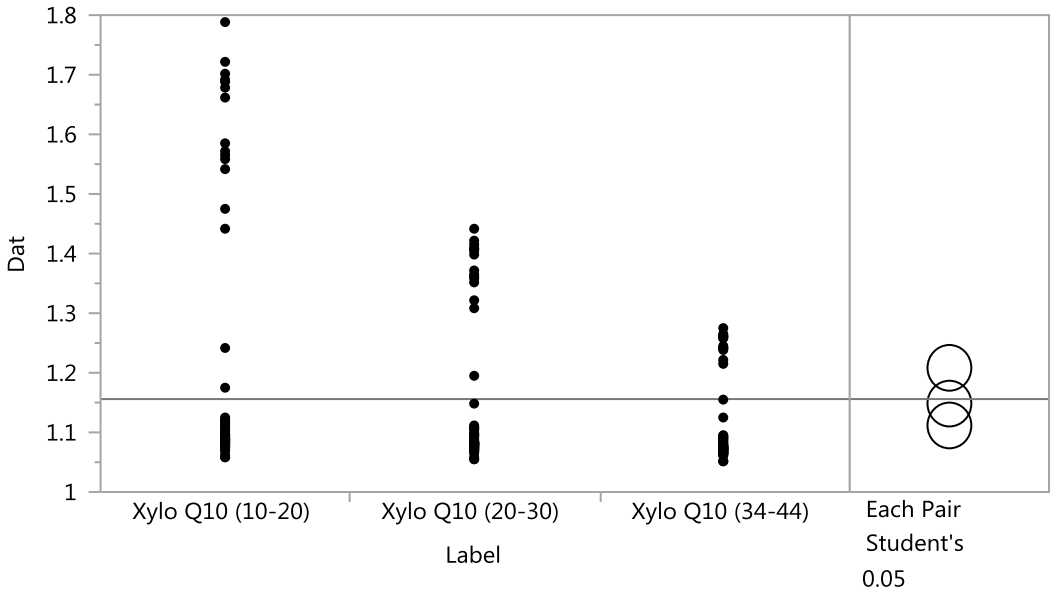
Figure 50. Temperature kinetics cellobiose response for three Q_{10} groups.



Connecting Letters Report

Level			Mean
Cello Q10 (10-20)	A		1.3763999
Cello Q10 (20-30)		B	1.2123166
Cello Q10 (34-44)		B	1.1464395

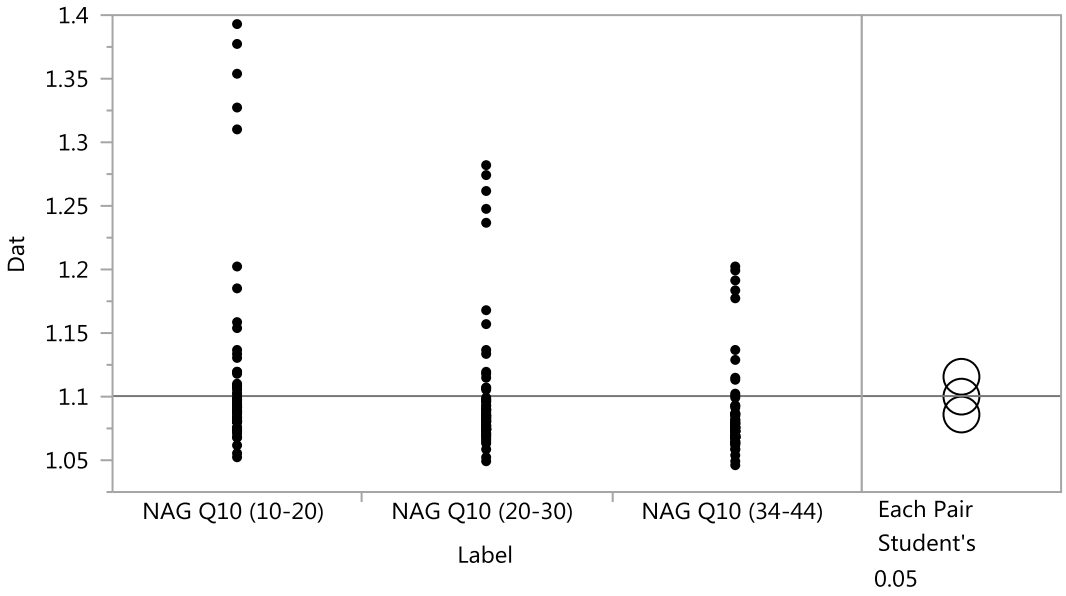
Figure 51. Temperature kinetics xylosidase response for three Q_{10} groups.



Connecting Letters Report

Level			Mean
Xylo Q10 (10-20)	A		1.2070491
Xylo Q10 (20-30)		B	1.1484451
Xylo Q10 (34-44)		B	1.1122859

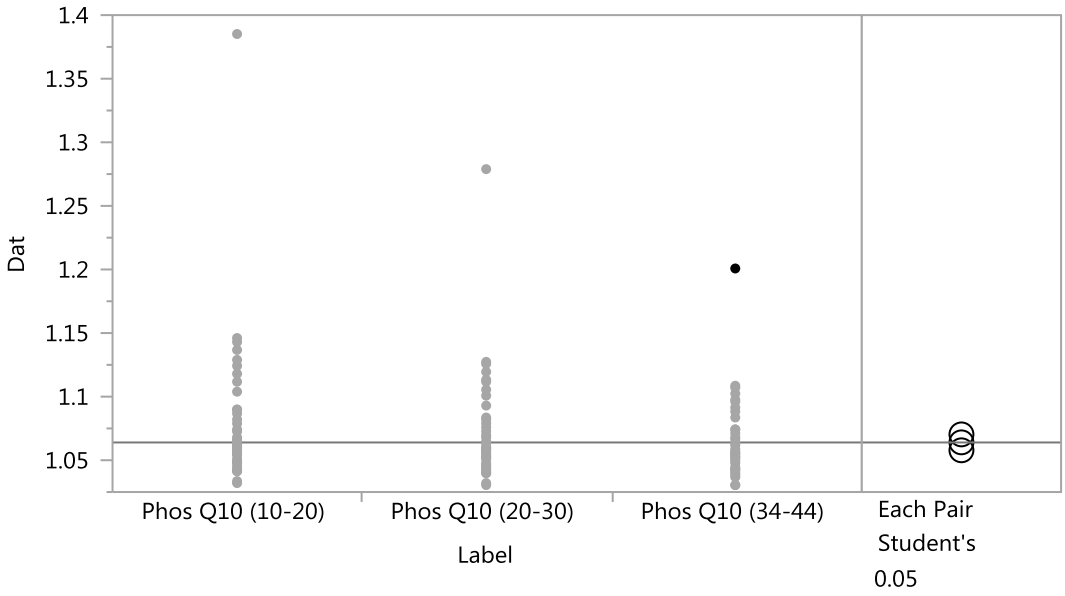
Figure 52. Temperature kinetic N-acteylglucosamine response for three Q_{10} groups.



Connecting Letters Report

Level			Mean
NAG Q10 (10-20)	A		1.1155249
NAG Q10 (20-30)	A	B	1.1001606
NAG Q10 (34-44)		B	1.0856663

Figure 53. Temperature kinetic phosphatase response for three Q_{10} groups.



Connecting Letters Report

Level		Mean
Phos Q10 (10-20)	A	1.0702562
Phos Q10 (20-30)	A	1.0640591
Phos Q10 (34-44)	A	1.0575997

Figure 54. A Hawaiian map of site locations for this study.

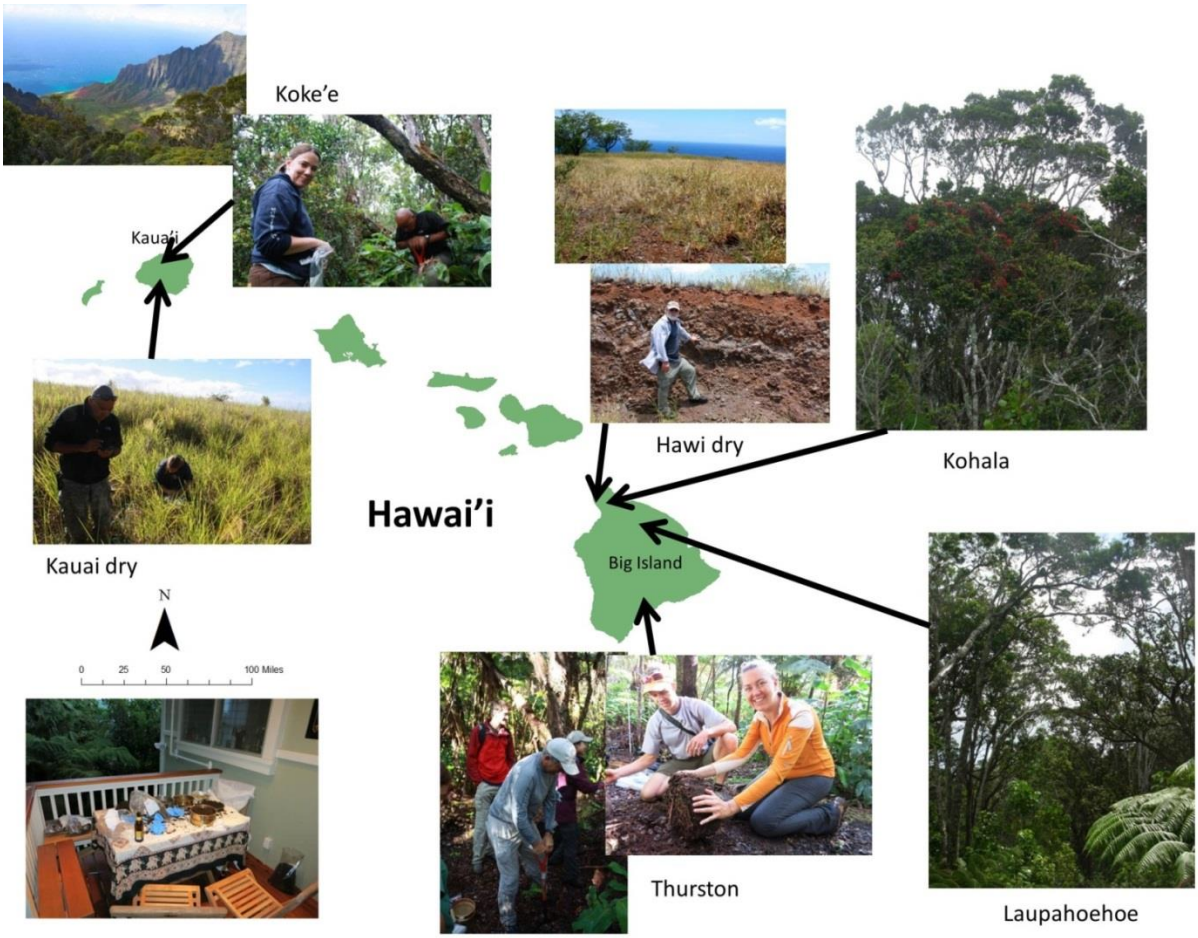


Table 1. Site information and nutrient composition is shown for six forest sites and two grasslands in Hawaii. Sites are ordered from lowest nutrient availability to highest.

Site	Relative Soil Nitrogen & Phosphorus	Field Fertilization	Extractable Inorganic N µg/g-soil	Bray P µg/g-soil	Relative Soil Carbon	Relative Soil Short Range Order Minerals	Vegetation Type	Precipitation mm/yr
Thurston Control	Low NP	no	10	2	Moderate	Low	Forest	2500
Kauai Dry	Low N (Mod. P)	no	9	58	High	Low	Grass	632
Koke'e Control	Low P (Mod. N)	no	30	1	High	Moderate	Forest	2500
Hawi Dry	Moderate NP	no	28	53	Low	Low	Grass	270
Thurston +NP	High P (Mod. N)	yes	10	99	Moderate	Low	Forest	2500
Koke'e +NP	High N (Mod. P)	yes	44	8	High	Moderate	Forest	2500
Laupahoehoe Control	High N (Mod. P)	no	146	11	High	High	Forest	2500
Kohala	High P (Mod. N)	no	30	45	High	High	Forest	2500

Table 2. Enzyme assays conducted on soil samples collected from Hawaii site. The enzyme names are followed by the substrate used in our assays.

Enzyme	Substrate	Function
Phenol Oxidase	L-3, 4-Dihydroxyphenylalanine	Oxygen catalyst
Peroxidase	L-3, 4-Dihydroxyphenylalanine	Oxygen catalyst
α-1, 4-Glucosidase	4-MUB-a-D-glucoside	Releases glucose from soluble saccharides
β-1, 4-Glucosidase	4-MUB-a-D-glucoside	Releases glucose from cellulose
Cellobiohydrolase	4-MUB- β-D-cellobioside	Releases disaccharides from cellulose
β -1, 4-N-Acetylglucosaminidase	4-MUB-N-acetyl β-D-Glucosaminide	Degrades chitin
Alkaline phosphatase	4-MUB- phosphate	Releases phosphate from phosphoric acid
B-1,4-Xylosidase	4-MUB- β-D-xyloside	Degrades hemi-cellulose

Table 3. Enzyme responses in forest sites versus grasslands for laboratory fertilized soils.

Precip	Ox	SE	Perox	SE	A-Gluc	SE	B-Gluc	SE	Cello	SE	Xylo	SE	NAG	SE	Phos	SE
270	148.7	28.2	144.4	24.1	148.5	13.2	1066.2	75.4	289.3	37.3	177.4	17.8	1152.2	104.3	3513.7	281.1
632	165.8	34.1	326.9	58.2	329.5	52.5	2559.2	321.6	846.3	139.5	280.2	45.8	1271.9	144.0	4267.2	668.6
2500	2356.6	369.0	2842.4	227.9	761.3	39.4	6801.4	233.3	2706.6	112.9	2449.3	93.8	3585.7	111.5	13225.0	537.0

Table 4. Enzyme activities across a rainfall gradient for laboratory fertilized soils (n=8).

Veg	Ox	SE	Perox	SE	A-Gluc	SE	B-Gluc	SE	Cello	SE	Xylo	SE	NAG	SE	Phos	SE
Forest	2356.6	369.0	2842.4	227.9	761.3	39.4	6801.4	233.3	2706.6	112.9	2449.3	93.8	3585.7	111.5	13225.0	536.982
Grass	157.2	22.0	235.7	32.4	239.0	28.1	1812.7	177.4	567.8	76.0	228.8	24.9	1212.0	88.7	3890.5	362.749

Table 5. Summary statistics for enzyme responses to laboratory nutrient fertilization (n=16).

Site Name	Fert	Oxi	SE	Perox	SE	A-Gluc	SE	B-Gluc	SE	Cello	SE	Xylo	SE	NAG	SE	Phos	SE
Hawi	C	82.4	24.3	64.6	20.0	73.4	7.5	604.0	51.7	109.8	17.9	97.1	4.9	475.7	46.4	2087.6	79.2
Hawi	N	0.0	0.0	0.0	0.0	217.7	9.6	1663.9	97.7	429.0	50.9	304.5	44.9	1635.9	96.6	5666.0	238.7
Hawi	NP	302.0	76.6	318.3	52.4	268.2	14.4	1478.3	103.8	531.2	100.3	251.8	20.1	2141.8	114.2	5390.0	360.5
Hawi	P	210.2	57.4	194.9	51.3	34.6	5.9	518.4	55.3	87.2	18.9	56.3	3.7	355.3	67.7	911.2	63.7
Kaui PM	C	47.2	21.2	234.3	47.3	29.9	3.5	482.8	46.1	40.1	23.5	33.8	6.7	595.6	43.7	301.4	33.5
Kaui PM	N	143.3	35.9	1073.5	51.3	697.2	148.6	3568.4	587.4	1356.6	329.0	537.0	133.5	2199.1	437.4	13070.7	435.1
Kaui PM	NP	423.2	105.4	0.0	0.0	551.8	16.4	5790.2	159.3	1968.9	166.8	530.2	16.0	1787.4	92.4	3299.6	204.5
Kaui PM	P	49.4	19.5	0.0	0.0	39.1	3.1	395.6	43.2	19.8	4.6	20.0	2.1	505.5	42.3	397.1	27.0
Kohala	C	21906.0	6637.4	0.0	0.0	295.7	35.2	4135.2	159.5	1734.6	131.8	2292.9	142.0	3345.1	179.4	10548.9	318.6
Kohala	N	6386.1	1260.5	59.6	38.1	542.7	36.6	6062.6	372.0	2343.5	188.0	2723.3	142.5	4570.4	185.4	23409.9	772.6
Kohala	NP	4343.3	1065.2	4176.7	1079.5	790.4	34.6	6949.0	329.3	3076.9	204.3	2871.9	228.9	4120.6	163.3	9580.6	752.6
Kohala	P	1394.2	591.4	7757.4	602.9	318.5	23.5	5582.1	317.5	1962.4	182.1	2664.5	169.9	4240.4	176.6	11582.9	479.3
Kokee Cont	C	58.6	23.2	539.1	67.4	31.0	3.7	1155.0	82.8	257.3	31.3	408.7	40.2	913.5	65.7	8220.3	221.2
Kokee Cont	N	61.2	27.8	542.8	84.1	1923.3	176.7	4485.8	187.0	2405.4	164.9	1166.9	108.4	2093.4	129.9	16742.4	347.8

Kokee Cont	NP	35.5	11.1	996.8	96.6	1707.6	84.7	6290.4	214.9	2448.2	350.6	1207.8	95.2	4254.4	505.9	6376.2	220.9
Kokee Cont	P	912.0	235.8	287.0	76.7	98.3	15.9	1513.0	176.3	371.6	43.0	630.2	53.3	1063.1	93.3	6895.4	248.3
Kokee NP	C	0.0	0.0	41.0	16.0	160.0	8.6	8646.6	465.2	3028.2	233.7	2636.5	159.6	1387.7	76.3	2394.3	105.2
Kokee NP	N	22.3	10.5	291.0	73.2	1111.5	62.4	16900.1	692.7	9277.2	363.7	3869.4	150.3	7475.6	280.0	9862.8	335.4
Kokee NP	NP	0.0	0.0	45.1	14.5	805.1	26.9	12238.1	384.5	5622.4	178.7	1936.9	127.4	3363.5	161.9	2232.0	92.1
Kokee NP	P	574.8	148.9	48.1	19.8	127.4	10.0	9081.6	667.3	2872.4	249.4	2713.5	196.5	1208.0	76.4	2125.4	148.7
Laup	C	2262.5	775.6	12552.2	753.1	1451.6	139.2	10083.0	703.5	4560.8	310.9	5851.9	282.0	4068.8	404.6	32317.3	1327.0
Laup	N	7174.1	1854.7	7236.3	570.9	1512.5	69.1	13344.6	864.9	5612.8	351.5	5707.7	222.3	6183.1	786.7	35348.6	2198.2
Laup	NP	717.2	139.8	13005.9	638.3	2994.1	127.9	14131.2	401.0	5129.9	202.4	5803.7	301.7	9264.9	282.8	28063.0	789.3
Laup	P	599.4	85.3	12454.8	189.0	1462.0	87.2	11771.7	686.6	3931.0	148.9	5808.4	287.3	4659.6	214.6	22337.6	1756.1
Thur Cont	C	0.0	0.0	3047.5	162.6	212.3	13.5	3198.6	194.6	1011.6	108.9	1398.4	111.6	2954.9	184.2	14957.5	695.6
Thur Cont	N	1362.1	34.0	993.3	122.8	413.7	26.2	4759.6	246.8	2031.3	162.6	1911.3	97.4	3083.9	139.0	29845.9	1033.1
Thur Cont	NP	1515.1	48.7	816.8	64.1	639.0	26.5	8595.3	359.1	3049.3	253.7	2603.9	95.9	4389.8	172.0	16057.2	380.0
Thur Cont	P	1586.6	62.5	694.1	61.8	256.7	16.7	2952.0	141.7	1014.9	91.0	1387.3	87.3	2803.1	136.8	12754.6	395.0
Thur NP	C	1876.2	214.4	792.8	104.1	112.9	13.6	2087.3	193.8	476.2	64.0	612.3	45.3	2694.2	218.4	3764.5	189.3
Thur NP	N	1095.2	20.8	477.3	46.1	650.1	21.3	4344.9	146.1	1679.1	124.6	1089.0	79.4	3655.3	161.8	4441.6	582.9
Thur NP	NP	1174.9	44.6	659.5	49.9	573.0	43.0	3783.0	217.8	1118.7	80.9	963.0	93.7	2578.3	91.8	4687.0	162.9
Thur NP	P	1500.8	35.0	703.2	40.5	104.8	7.0	1773.5	115.8	352.5	40.8	613.1	43.3	1927.7	96.0	2644.4	141.4

Table 6. Summary statistics for enzyme responses to temperature (n=8).

Site Name	Temp (°C)	Oxi	SE	Perox	SE	A-Gluc	SE	B-Gluc	SE	Cello	SE	Xylo	SE	NAG	SE	Phos	SE
Hawi	4	125.0	7.9	37.5	5.2	0.0	0.0	28.7	5.8	0.2	0.2	0.2	0.2	27.7	18.0	64.3	7.5
Hawi	10	92.8	3.7	38.6	8.5	5.2	0.4	39.6	3.4	9.1	3.0	6.5	0.8	18.8	1.9	59.8	2.3
Hawi	16	93.2	8.3	6.4	5.6	9.4	0.4	76.8	3.4	0.7	0.4	17.5	6.7	48.9	7.2	173.8	48.7
Hawi	22	125.2	6.8	0.0	0.0	16.9	3.2	200.4	9.6	34.7	12.5	16.0	1.0	79.8	19.8	144.2	4.0
Hawi	27	167.5	18.3	112.8	15.3	42.4	4.7	263.0	33.2	57.4	17.7	57.7	9.1	216.4	62.1	197.5	37.6
Hawi	34	335.3	9.1	92.2	10.5	20.4	1.2	278.8	117.8	32.7	10.5	28.5	8.3	77.8	4.5	175.3	5.1
Hawi	40	632.6	33.6	43.9	30.1	357.0	38.5	153.2	26.6	115.8	20.6	114.1	21.6	349.9	77.4	469.9	39.5
Kauai PM	4	39.9	14.1	0.0	0.0	0.0	0.0	40.6	8.3	1.0	1.0	2.0	2.0	69.9	5.8	79.5	14.4
Kauai PM	10	1.3	1.3	138.5	8.5	4.9	0.9	101.6	5.7	7.6	0.9	11.8	1.0	62.3	2.0	120.3	4.0
Kauai PM	16	0.0	0.0	144.1	16.5	14.4	2.9	140.6	2.8	1.5	1.0	26.6	1.7	103.8	3.6	289.6	74.5
Kauai PM	22	0.0	0.0	0.0	0.0	15.5	1.4	173.3	14.8	28.9	2.6	40.7	4.2	190.3	14.3	243.9	40.1
Kauai PM	27	5.8	3.7	0.1	0.1	31.0	9.1	231.4	106.4	64.8	17.0	54.6	3.8	227.8	21.7	337.8	179.4
Kauai PM	34	0.0	0.0	0.0	0.0	28.3	2.4	192.8	18.9	42.5	4.8	56.7	7.3	379.7	51.0	220.1	61.7
Kauai PM	40	276.3	22.0	0.0	0.0	48.3	11.9	145.2	9.7	131.9	28.4	128.8	35.1	310.2	18.9	283.2	13.4
Kohala	4	0.0	0.0	0.0	0.0	79.0	31.7	1262.6	141.9	178.4	25.4	456.2	21.2	855.0	63.7	4886.0	229.2
Kohala	10	0.5	0.5	132.2	7.6	176.5	51.9	1920.8	269.6	560.5	136.4	1078.4	125.1	1258.8	32.6	5148.9	92.7
Kohala	16	0.6	0.6	315.8	12.7	379.0	13.7	3238.3	165.1	1280.9	175.9	1654.7	131.8	2441.4	86.7	9179.8	182.8
Kohala	22	31.4	10.1	20.5	5.7	489.3	19.1	5304.5	160.1	1399.3	118.1	2800.4	285.9	2986.5	442.0	10906.7	216.9
Kohala	27	9.5	5.5	381.3	10.3	782.3	65.4	5409.5	418.2	1923.7	152.8	1921.0	149.0	5279.1	456.0	13851.3	539.8
Kohala	34	79.1	6.3	210.2	19.4	857.9	93.7	6848.5	247.9	2797.1	288.7	3095.3	166.4	5889.6	374.5	14270.5	620.7
Kohala	40	273.8	11.3	248.0	11.6	367.3	36.5	4277.2	685.7	5069.6	867.5	4595.8	293.9	7747.7	338.4	20129.7	2171.4
Kokee Cont	4	0.0	0.0	0.0	0.0	8.7	3.7	361.6	39.1	43.6	8.6	107.8	7.8	299.4	24.6	3537.8	153.9
Kokee Cont	10	0.0	0.0	0.0	0.0	41.9	6.9	960.5	82.8	231.2	40.3	286.3	25.1	501.8	34.4	4528.2	193.8
Kokee Cont	16	25.5	11.9	28.3	7.9	135.8	8.2	1628.2	115.4	591.9	93.3	518.5	72.4	841.2	55.0	7382.5	131.7
Kokee Cont	22	194.9	6.3	10.8	7.0	162.3	8.1	2633.1	104.4	698.8	107.4	657.4	124.6	1334.5	49.3	9233.8	206.5

Kokee Cont	27	16.8	7.8	168.8	21.9	185.0	31.9	1662.4	290.1	350.1	59.5	405.8	60.6	1003.5	165.6	8436.2	1259.0
Kokee Cont	34	0.0	0.0	0.0	0.0	271.9	17.0	2261.6	145.0	953.4	97.7	987.2	77.0	1836.1	80.9	11837.7	335.7
Kokee Cont	40	191.8	7.9	180.1	14.5	110.0	4.3	1365.4	63.3	772.7	127.0	880.4	47.3	1796.1	188.8	12982.0	1943.6
Kokee NP	4	13.9	13.9	0.0	0.0	6.5	0.8	2271.2	98.5	469.0	19.2	682.3	21.1	259.0	26.3	337.7	79.9
Kokee NP	10	0.0	0.0	0.0	0.0	39.2	2.2	4495.0	163.4	1238.1	82.2	1436.3	62.8	567.4	37.6	837.1	53.0
Kokee NP	16	0.0	0.0	3.3	3.1	123.1	3.9	6869.9	193.4	2581.7	191.1	2337.8	56.7	865.7	24.5	1860.1	59.8
Kokee NP	22	0.0	0.0	0.0	0.0	152.2	7.8	5596.3	131.4	3416.0	182.9	2768.8	67.5	1467.0	80.8	2065.8	89.5
Kokee NP	27	0.0	0.0	193.5	5.6	320.8	16.0	12284.7	439.7	3027.1	54.9	2612.0	70.4	2244.4	199.9	2682.2	105.0
Kokee NP	34	0.0	0.0	86.4	10.7	728.3	203.0	16500.4	823.8	5454.4	134.8	8262.5	390.1	2939.1	193.0	3442.2	145.1
Kokee NP	40	0.0	0.0	0.0	0.0	131.8	9.3	9802.8	1377.3	8915.6	252.8	6009.2	256.8	3167.6	283.3	3057.6	719.8
Laup	4	0.0	0.0	0.0	0.0	42.8	10.1	3785.8	429.8	452.4	64.7	1531.3	121.4	1795.3	374.0	18268.7	2089.1
Laup	10	159.9	23.5	368.7	11.5	152.8	15.1	5872.0	122.1	1287.3	141.1	2639.2	60.4	1513.8	102.3	18310.4	267.8
Laup	16	38.1	18.4	648.7	13.9	589.3	26.8	10529.7	461.7	2771.0	117.9	4677.5	217.4	3112.1	268.2	27497.3	823.5
Laup	22	319.2	8.9	0.0	0.0	755.2	38.0	9236.2	439.4	3644.9	185.0	5564.3	244.6	4517.3	448.0	32889.8	1152.0
Laup	27	549.4	8.9	500.0	17.9	1289.0	71.0	17025.4	1021.2	4021.8	190.0	5558.4	436.8	4911.7	276.1	49905.1	2945.2
Laup	34	315.9	10.8	427.7	18.4	1309.7	76.1	19576.2	1226.6	6143.2	386.4	8724.3	397.9	7805.1	527.0	54862.8	3153.2
Laup	40	6467.3	820.2	0.0	0.0	806.6	54.8	13106.8	1890.2	10901.6	564.0	12621.6	597.0	10310.0	840.4	70483.8	6282.5
Thur Cont	4	0.0	0.0	77.6	4.3	46.5	1.7	820.2	49.8	188.2	13.0	423.5	19.9	1030.3	99.2	5516.6	230.7
Thur Cont	10	4.2	4.2	91.0	7.8	57.5	2.7	1406.7	63.2	380.6	42.0	602.5	22.7	1294.5	78.5	5774.2	103.3
Thur Cont	16	22.6	7.2	231.0	6.4	242.6	5.5	2897.8	224.5	836.2	48.9	1103.0	65.0	2564.2	133.8	9924.0	237.5
Thur Cont	22	162.2	9.9	0.0	0.0	316.6	5.1	2902.2	139.5	1147.3	92.3	1484.4	88.5	151.8	5.3	12507.4	367.8
Thur Cont	27	207.6	22.3	54.6	12.1	461.6	7.0	6161.9	357.1	1248.9	89.4	1460.5	38.8	4500.4	195.1	19857.2	335.6
Thur Cont	34	224.7	17.6	122.8	10.1	553.9	41.0	6423.0	436.1	1465.0	69.1	1920.0	103.2	5142.6	109.0	20147.5	806.6
Thur Cont	40	365.5	13.2	13.0	7.0	260.1	16.2	4601.1	765.1	3210.0	243.1	3689.6	280.3	9526.7	421.9	36418.6	1219.1
Thur NP	4	12.9	7.5	158.6	9.7	19.0	0.9	566.2	25.3	85.9	9.8	200.4	8.1	665.7	29.5	1687.0	42.8
Thur NP	10	28.6	3.3	140.1	14.5	49.5	1.2	944.0	58.3	286.6	32.9	337.9	19.6	1278.3	127.4	2122.0	46.7
Thur NP	16	0.0	0.0	1839.8	105.2	161.3	3.2	1885.3	93.2	514.6	60.4	579.9	27.2	2147.2	121.0	4158.1	57.4
Thur NP	22	120.0	9.7	0.0	0.0	219.7	3.3	2764.0	189.4	588.9	42.3	760.7	39.9	602.9	307.5	4540.9	140.9
Thur NP	27	137.1	10.5	137.5	16.1	257.6	4.8	2929.6	178.8	739.7	89.8	745.5	31.8	3449.7	67.0	6203.7	165.3
Thur NP	34	229.4	8.7	28.3	8.3	355.0	21.0	4166.5	212.5	1149.8	102.4	1963.1	110.2	5343.4	193.2	7051.1	248.8
Thur NP	40	295.2	10.7	103.3	17.1	141.5	8.8	2271.9	325.4	1679.5	99.1	1968.8	88.1	6879.6	183.2	9439.9	1364.4

Table 7. Summary statistics for Q10 kinetics by enzyme and site (n=8). * Slopes were recalculated from 34-44° C to account for decreased enzyme activity for alpha-glucosidase and beta-glucosidase.

	Site Name	Intercept	SE	Slope	SE	Intercept*	SE	Slope*	SE	Q10-20	SE	Q20-30	SE	Q34-44	SE
Oxidative	Hawi	4.10	0.06	0.05	0.00					1.11	0.00	1.10	0.00	1.08	0.00
	Kauai	0.22	0.30	0.05	0.01					1.38	0.48	1.53	0.17	1.27	0.04
	Kohala	-1.42	0.13	0.16	0.01					4.39	4.13	1.92	0.04	1.40	0.01
	Koke'e +NP	0.00	0.00	0.00	0.00					0.00	0.00	0.00	0.00	0.00	0.00
	Koke'e Control	-0.63	0.68	0.09	0.00					2.28	0.41	1.42	0.13	1.15	0.15
	Laupahoehoe	0.66	0.23	0.19	0.00					1.78	0.07	1.43	0.02	1.27	0.01
	Thurston +NP	0.82	0.36	0.13	0.01					1.71	0.13	1.39	0.05	1.25	0.02
	Thurston Control	-0.74	0.16	0.18	0.01					3.12	0.42	1.64	0.04	1.34	0.01
Peroxidative	Hawi	2.47	0.24	0.01	0.01					1.04	0.05	1.02	0.05	1.00	0.05
	Kauai	3.32	0.05	-0.09	0.00					0.64	0.00	0.45	0.00	-1.46	0.07
	Kohala	2.01	0.08	0.10	0.00					1.34	0.01	1.25	0.01	1.19	0.00
	Koke'e +NP	-0.25	0.11	0.08	0.00					2.67	0.19	1.61	0.04	1.33	0.01
	Koke'e Control	-0.23	0.18	0.09	0.01					4.88	2.23	1.63	0.07	1.33	0.02
	Laupahoehoe	3.58	0.01	0.00	0.00					0.99	0.00	0.99	0.00	0.99	0.00
	Thurston +NP	5.46	0.16	-0.06	0.01					0.88	0.02	0.86	0.03	0.81	0.06
	Thurston Control	4.80	0.11	-0.06	0.01					0.85	0.03	0.82	0.04	0.73	0.06
Alpha-Glucosidase	Hawi	0.35	0.04	0.22	0.11	-12.79	0.45	0.47	0.01	1.78	0.04	1.43	0.01	2.53	0.05
	Kauai	0.31	0.11	0.10	0.00	0.95	1.27	0.07	0.04	1.81	0.08	1.44	0.02	1.23	0.12
	Kohala	4.11	0.24	0.09	0.01	11.56	0.76	-0.37	0.24	1.18	0.02	1.15	0.02	0.87	0.08
	Koke'e +NP	2.00	0.12	0.14	0.01	15.31	1.34	0.91	1.18	1.41	0.03	1.29	0.01	0.67	0.09
	Koke'e Control	2.25	0.29	0.11	0.01	10.65	0.49	-0.14	0.01	1.34	0.06	1.25	0.03	0.75	0.02
	Laupahoehoe	3.76	0.18	0.12	0.01	9.92	0.42	-0.08	0.01	1.24	0.02	1.19	0.01	0.89	0.02
	Thurston +NP	3.02	0.04	0.09	0.00	11.07	0.44	-0.15	0.01	1.24	0.01	1.19	0.00	0.74	0.02
	Thurston Control	3.57	0.04	0.09	0.00	10.56	0.73	-0.13	0.02	1.20	0.01	1.17	0.00	0.80	0.03
Beta-Glucosidase	Hawi	3.07	0.17	0.08	0.01	7.70	2.91	-0.07	0.08	1.21	0.03	1.17	0.02	0.94	0.07
	Kauai	3.92	0.12	0.04	0.01	6.76	0.67	-0.04	0.02	1.10	0.03	1.09	0.02	0.95	0.02
	Kohala	7.01	0.08	0.06	0.00	12.90	1.97	-0.12	0.06	1.08	0.00	1.07	0.00	0.92	0.04

	Koke'e +NP	7.63	0.03	0.06	0.00	14.02	2.16	-0.13	0.06	1.07	0.00	1.07	0.00	0.92	0.04
	Koke'e Control	6.10	0.07	0.06	0.00	10.53	0.51	-0.08	0.01	1.09	0.01	1.08	0.00	0.94	0.01
	Laupahoehoe	7.11	0.97	0.05	0.00	13.64	2.16	-0.11	0.06	1.12	0.06	1.10	0.04	0.93	0.04
	Thurston +NP	6.23	0.03	0.07	0.00	13.30	2.22	-0.15	0.07	1.10	0.00	1.09	0.00	0.89	0.05
	Thurston Control	6.56	0.07	0.07	0.00	12.11	2.11	-0.10	0.06	1.10	0.00	1.09	0.00	0.93	0.04
Cellobiose	Hawi	-0.02	0.16	0.12	0.01					2.13	0.19	1.51	0.04	1.29	0.01
	Kauai	0.05	0.24	0.12	0.01					2.08	0.15	1.50	0.04	1.29	0.02
	Kohala	5.27	0.17	0.08	0.01					1.14	0.01	1.12	0.01	1.10	0.01
	Koke'e +NP	6.27	0.04	0.07	0.00					1.10	0.00	1.09	0.00	1.08	0.00
	Koke'e Control	4.39	0.12	0.07	0.00					1.14	0.01	1.12	0.01	1.10	0.00
	Laupahoehoe	6.21	0.06	0.08	0.00					1.11	0.00	1.10	0.00	1.09	0.00
	Thurston +NP	4.64	0.08	0.07	0.00					1.14	0.01	1.12	0.01	1.10	0.00
	Thurston Control	5.24	0.05	0.16	0.09					1.18	0.06	1.14	0.03	1.11	0.02
Xylosidase	Hawi	0.53	0.07	0.10	0.00					1.67	0.03	1.40	0.01	1.26	0.00
	Kauai	1.23	0.24	0.09	0.01					1.46	0.06	1.31	0.03	1.21	0.02
	Kohala	6.26	0.04	0.06	0.00					1.08	0.00	1.08	0.00	1.07	0.00
	Koke'e +NP	6.53	0.03	0.06	0.00					1.09	0.00	1.08	0.00	1.07	0.00
	Koke'e Control	4.95	0.09	0.05	0.00					1.10	0.01	1.09	0.00	1.08	0.00
	Laupahoehoe	7.31	0.05	0.05	0.00					1.07	0.00	1.06	0.00	1.06	0.00
	Thurston +NP	5.25	0.10	0.06	0.00					1.11	0.00	1.10	0.00	1.09	0.00
	Thurston Control	5.92	0.03	0.05	0.00					1.08	0.00	1.08	0.00	1.07	0.00
N-acetylglucosidase	Hawi	2.28	0.25	0.08	0.01					1.28	0.04	1.22	0.02	1.16	0.01
	Kauai	3.90	0.07	0.05	0.00					1.12	0.01	1.11	0.01	1.09	0.01
	Kohala	6.63	0.04	0.06	0.00					1.09	0.00	1.08	0.00	1.07	0.00
	Koke'e +NP	5.55	0.09	0.07	0.00					1.11	0.01	1.10	0.00	1.09	0.00
	Koke'e Control	5.75	0.04	0.05	0.00					1.08	0.00	1.07	0.00	1.07	0.00
	Laupahoehoe	7.02	0.13	0.06	0.00					1.07	0.01	1.07	0.01	1.06	0.00
	Thurston +NP	6.45	0.06	0.06	0.00					1.09	0.00	1.08	0.00	1.07	0.00
	Thurston Control	6.69	0.06	0.06	0.00					1.08	0.00	1.08	0.00	1.07	0.00
Phosphatase	Hawi	3.82	0.08	0.05	0.00					1.12	0.01	1.11	0.01	1.09	0.00
	Kauai	4.46	0.09	0.03	0.00					1.07	0.01	1.06	0.01	1.06	0.01
	Kohala	8.32	0.04	0.04	0.00					1.05	0.00	1.04	0.00	1.04	0.00
	Koke'e +NP	5.86	0.44	0.07	0.02					1.12	0.04	1.10	0.03	1.08	0.02

Koke'e Control	8.12	0.03	0.04	0.00		1.05	0.00	1.04	0.00	1.04	0.00
Laupahoehoe	9.54	0.07	0.04	0.00		1.04	0.00	1.04	0.00	1.04	0.00
Thurston +NP	7.29	0.01	0.05	0.00		1.06	0.00	1.06	0.00	1.06	0.00
Thurston Control	8.30	0.03	0.05	0.00		1.06	0.00	1.06	0.00	1.05	0.00

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