Impact of nitrogen availability and nitrogen structural composition on fungal enzymatic activity and growth

How nutrient availability governs response and development of three saprotrophic Basidiomycetes

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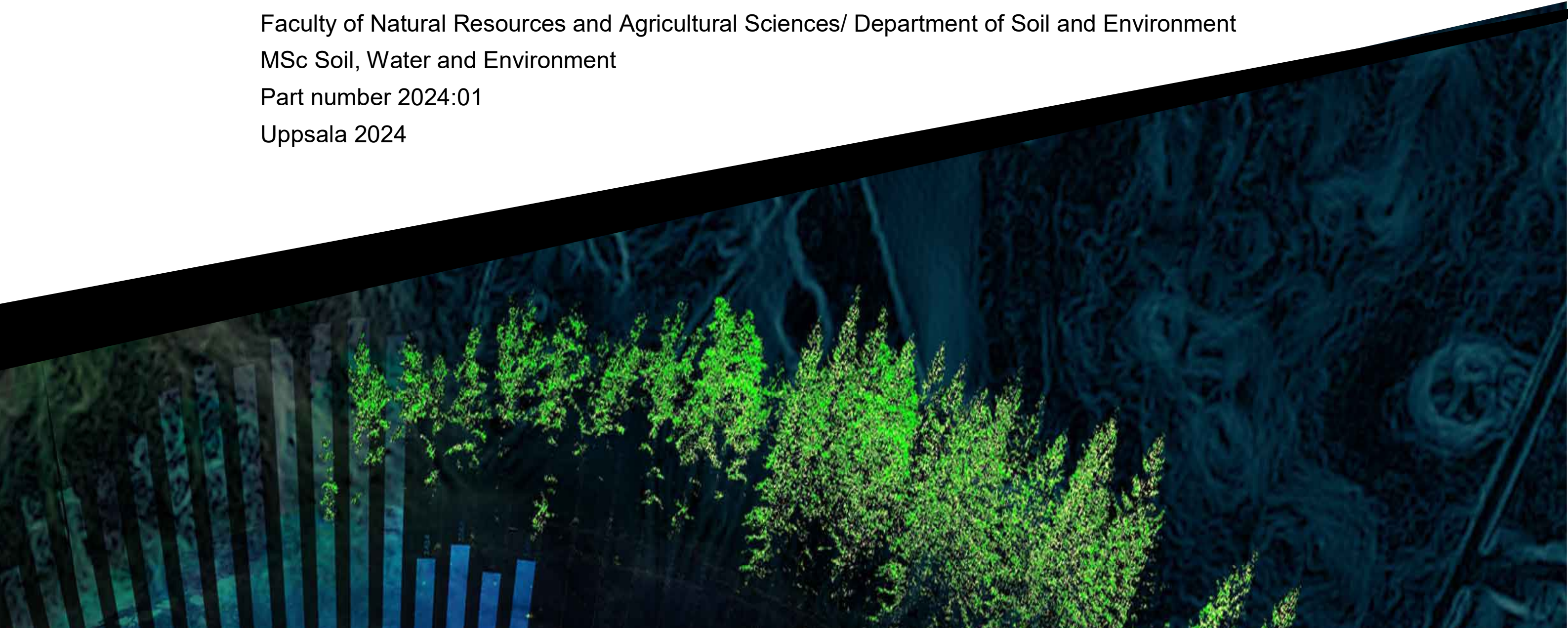

Impact of nitrogen availability and nitrogen structural compositon on fungal enzymatic activity and growth. How nutrient availability governs fungal response and development

Inverkan av kvävetillgänglighet och kvävestrukturell sammansättning på svampens enzymatiska aktivitet och tillväxt. Hur näringstillgänglighet styr svamprespons och utveckling.

Bella Strid

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Abstract

Fungi excrete a wide variety of extracellular enzymes to scavenge for nutrients, such as the often scarce yet essential nutrient nitrogen. All fungi produce highly specialized hydrolytic enzymes, e.g. peptidases, that depolymerize organic molecules. However, some organic matter such as lignin and tannins largely lack the nitrogen or oxygen bridges required for hydrolysis, and these recalcitrant structures thus require a different approach for decomposition in order to reach the nitrogen within. Certain fungal species have the capacity to produce highly reactive and unspecific peroxidases which free nutrients tied to these plant secondary metabolites. Peroxidases can break strong C-C bonds through electron transfer and efficiently catabolize unhydrolyzable compounds. Saprotrophic basidiomycetes are considered our main decomposers of recalcitrant organic matter in soils due to their ability to use manganese peroxidases and are key contributors to the cycling of carbon and nitrogen. These nutrient cycles are highly interlinked, which is why some forests are fertilized with nitrogen to boost plant biomass production and thus carbon sequestration, in attempts to decrease the greenhouse gas effect. As plants rely less on symbiosis with microorganisms while non-symbiotic microorganisms may benefit from increased nitrogen availability, fertilization is likely to have implications on fungal biodiversity. I investigated if and how three species of saprotrophic basidiomycetes adapt their peptidase and manganese peroxidase activity to varying sources of nitrogen and how it affects their biomass production. Laboratory experiments were conducted where the fungi were provided high and low concentrations of readily available mineral nitrogen, easily accessible organic nitrogen, recalcitrant nitrogen as tannin-protein complexes, or given no nitrogen at all. Manganese peroxidases and peptidases were sampled weekly for three weeks and analyzed colorimetrically and through fluorescence spectroscopy, respectively. Biomass was measured at the end of the experiment. Data was analyzed with Repeated Measures ANOVA and TukeyHSD. Recalcitrant nitrogen was expected to trigger high levels of manganese peroxidase activity, organic nitrogen to trigger increased peptidase, while mineral nitrogen was expected to cause no significant activity of either enzyme. All fungi were predicted to gain largest biomass when provided mineral nitrogen, and smallest biomass when provided no nitrogen at all.

The three species differed in their responses, none of which fully met expectations. Generally, nutrient source did not affect enzymatic activity, but it was most often affected by what concentration of respective source it was given. Trends of enzyme activities over time was often similar for a fungus between concentrations of the same nitrogen source, but what these trends looked like varied between species. Growth seems in some cases to be correlating with levels of manganese peroxidase activity, where higher manganese peroxidase activity perhaps came at a cost of lower biomass production. Biomass responses varied between species where some benefitted from mineral nitrogen while others yielded greater biomass given recalcitrant organic matter. Such varying responses points to how challenging it is to forecast changes in community compositions and ecosystem function following anthropogenic interference.

Keywords: nitrogen, tannins, condensed tannins, MnP, manganese peroxidase, peptidase, saprotrophic basidiomycetes

Sammanfattning

Svampar utsöndrar ett brett utbud av extracellulära enzymer för att söka efter näringsämnen, såsom det ofta knappa men väsentliga näringsämnet kväve. Alla svampar producerar specialiserade hydrolytiska enzymer, t.ex. peptidaser, som depolymeriserar organiska molekyler. Vissa organiska ämnen, som lignin och tanniner, saknar i stor utsträckning de kväve- eller syrebroar som krävs för hydrolys, och dessa strukturer kräver därför ett annat tillvägagångssätt för nedbrytning för att nå kvävet inuti. Vissa svamparter har förmågan att producera högreaktiva och ospecifika peroxidas som frigör näringsämnen bundna till dessa sekundära växtpolyfenoler. Peroxidaser kan bryta starka C-C-bindningar genom elektronöverföring och effektivt katabolisera ohydrolyserbara föreningar. Saprotrofa basidiesvampar anses vara våra viktigaste nedbrytare av motsträvigt organiskt material i jord tack vare deras förmåga att använda manganperoxidas och är nyckelaktörer i kol- och kvävecykling. Dessa näringscykler är starkt sammanflätade, vilket är varför vissa skogar gödslas med kväve för att öka produktionen av växtbiomassa och därmed kolbindning, i ett försök att minska växthuseffekten. Eftersom växter förlitar sig mindre på symbios med mikroorganismer medan icke-symbiotiska mikroorganismer kan gynnas av ökad tillgänglighet av kväve, kan gödsling sannolikt ha konsekvenser för svampbiodiversiteten. Jag undersökte om och hur tre arter av saprotrofa basidiesvampar anpassar sin peptidas- och manganperoxidasaktivitet till varierande källor av kväve och hur det påverkar deras biomassaproduktion. Laboratorieexperiment utfördes där svamparna tillhandahölls höga och låga koncentrationer av lättillgängligt mineraliskt kväve, lättillgängligt organiskt kväve, motsträvigt kväve som tannin-protein-komplex, eller inget kväve alls. Manganperoxidas och peptidas provtogs veckovis i tre veckor och analyserades kolorimetriskt och genom fluorescensspektroskopi, respektive. Biomassan mättes i slutet av experimentet. Data analyserades med Repeated Measures ANOVA och TukeyHSD. Det förväntades att motsträvigt kväve skulle generera höga nivåer av manganperoxidasaktivitet, organiskt kväve generera ökad peptidas, medan mineraliskt kväve inte förväntades generera signifikant aktivitet av något enzym. Alla svampar förutspåddes få störst biomassaproduktion när de tillhandahölls mineraliskt kväve och minst biomassaproduktion när de inte tillhandahölls något kväve alls.

De tre arterna skilde sig åt i sin respons, inget av dem uppfyllde helt förväntningarna. Generellt påverkade näringskällan inte enzymaktiviteten, men den påverkades istället oftast av vilken koncentration av respektive kvävekälla den gavs. Trenderna i enzymaktiviteter över tid var ofta liknande för en svamp mellan koncentrationer av samma kvävekälla, men hur dessa trender såg ut varierade mellan arterna. Tillväxt verkar i vissa fall korrelera med nivåerna av manganperoxidasaktivitet, där högre manganperoxidasaktivitet eventuellt kom på bekostnad av lägre biomassaproduktion. Produktion av biomassa varierade mellan arter där vissa gynnades av mineraliskt kväve medan andra växte sig större på motsträvigt organiskt material. Sådana varierande resultat pekar på hur utmanande det är att förutspå förändringar i artsammansättning och ekosystemfunktion efter antropogen påverkan.

Nyckelord: kväve, tanniner, kondenserade tanniner, MnP, manganperoxidas, peptidas, saprotrofa basidiesvampar

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Abbreviations

CT	Condensed tannins
HE	Hydrolytic enzymes
Hypholoma	*Hypholoma fasciculare*
Mycena	*Mycena epipterygia*
Marasmius	*Marasmius oreades*
Mn	Manganese
MnP	Manganese peroxidase
N	Nitrogen
OE	Oxidative enzymes

1. Background/problem description

Life on Earth has evolved for billions of years, spawn from simple chemical bonds and interactions that followed universal laws of physics (Butch et al 2021). Random trials and repeated patterns led to reproduction, allowing an unbroken chain of serendipity to create a vast diversity of lifeforms, which constitutes all the existing ecosystems we have today. Although the kingdoms are incredibly rich in genetic coding and function, some things are common for the majority of life as we know it; carbon based life requires a set of elements to maintain functionality (Gwinnup & Schnoor 2014). As generations come and go, nutrients and building blocks pass through the system of an individual, and as living things turn dead, their atomic building blocks remain tied to the old body. It is of interest for microorganisms to break down this biotic matter to retrieve important substances (Madsen 2011), as this continuous flow of material through the system is what keeps life living. The cycles of our major essential elements oxygen, phosphorus, carbon and nitrogen are highly interlinked (Gruber & Galloway 2008) and as we battle an increase in atmospheric carbon dioxide (CO_2), it is vital to understand the interaction of these nutrient cycles. In attempts to decrease the greenhouse effect, efforts are made to increase soil carbon through carbon sequestration (Lal 2004, Goh 2004). A frequently used method is fertilization of boreal forests to enhance the vegetations ability to capture atmospheric carbon and store it within both living and deposited biomass (Lal 2004). Carbon sequestration success hinges on the long-term stability of soil carbon, but the impact of modified nutrient quantities is complex and may give unwanted side effects. Aside from stimulating plant growth, fertilizers also influence the composition and activity of microbiological communities (Demoling et al 2008, Dincă 2022), both of which play an important part in forest soil respiration and in organic matter turnover (Demoling et al 2008). As microbiological species fill different roles and functions within ecosystems and in nutrient pathways, they are inevitably affected quite differently by alterations of resource availability. While some are benefiting from disturbances such as fertilization, others are outcompeted, shifting community composition (Dincă 2022). Fungi associated with plants have been observed to diminish following nitrogen input; as their host plants no longer rely on them for nitrogen uptake, the supply of plant metabolites to these fungi consequently ceases (Jörgensen et al 2021). Meanwhile, fungi that acquire nutrients (particularly carbon) through other means than symbiosis, often increase. Some of these non-symbiotic fungal species exhibit remarkable efficiency in decomposing resistant, carbon-rich plant material to obtain nutrients, releasing surplus carbon through respiration (Cabrera et al 2005). Following fertilization, decomposition have been found to both increase, decrease and remain unaffected (Jörgensen et al 2021, Okal et al 2020, Voříšková et al 2011, Janssens et al 2010, Kirk & Tien 1988). If some fungal species prosper from fertilization, it is of interest to understand if and how they change their decomposition activity, as there may be a risk that an increase in nitrogen will increase wood-decomposition, thus counteracting carbon sequestration efforts. This invokes a necessity to better understand how wood-decomposing fungi in boreal forests respond to different forms and concentrations of nutrients, in order to tackle the ongoing climate crisis as well as to address the rapid decrease in biodiversity.

1.1 Nitrogen and secondary metabolites

Nitrogen (N) is a fundamental component in biochemistry, and the way organisms utilize nitrogen in their molecular structures have an important impact on the N cycle path (Kögel-Knabner 2002). About 78 % of the atmosphere consists of nitrogen (N_2), yet it is often a limiting factor for plants and microorganisms as only a few types of bacteria can bind gaseous nitrogen (Gruber & Galloway 2008, Burris & Roberts 1993). Through biosynthesis, N becomes part of larger molecular structures such as amino acids – the structural units of proteins and thus enzymes – and nucleic acids, which are the foundational components of genetic material (Berkeley 2023, Kögel-Knabner 2002). As a component ceases to serve an organism due to death or excretion, other microorganisms begin to systematically break the component down to utilize the building blocks (Madsen 2011). Occasionally, intact biomolecules may be directly utilized, but more frequently, the mineralization process results in simple inorganic compounds such as ammonium (NH_4^+) or nitrate (NO_3^-) (Cabrera et al 2005) which are subsequently immobilized.

Soil nitrogen is however not always easily accessed, as the effort required by microorganisms to retrieve soil nitrogen varies depending on structural associations of N. Primary metabolites like proteins, peptides and amines fill active life supporting functions in a body, such as growth, development and reproduction, and are essential for the organisms survival. By necessity, the composition of primary metabolites are highly predictable, precise, and repeated. Hence, catabolism is predictable as well, allowing organisms to use catabolic enzymes which are highly specific. Unlike primary metabolites, secondary metabolites are not directly involved in these life-essential processes but rather play a role in interactions with the environment. While primary metabolites are regular and repeated, secondary metabolites are often more complex and unpredictable, efficiently deterring external decomposition (Adamczyk et al 2017, Kögel-Knabner 2002). For example, plants protect their intracellular parts by metabolizing hard-to-digest structures, which help them protect their primary metabolites, as most organisms lack the capacity to break them down (Kögel-Knabner 2002). These structures deter microbial attack both by physical blockade and by strong chemical complexation with intracellular storage materials, making plant nutrients largely unavailable for acquisition.

There are a few varieties of plant tissues, two of which are lignin and tannin. Lignin, which constitutes a large proportion of wood, is a three-dimensional macromolecule containing a large amount of C-C bonds and ether-links making it largely resistant to hydrolysis, thus fending off most attempts of microbial decomposition. By interacting with plant primary metabolites containing nitrogen, lignin act as protection against nitrogen loss (Kögel-Knabner 2002). Tannins, another protective structure, are molecules consisting of multiple acidic hydroxyl (OH) groups, which are attached to phenyl groups that contain large amounts of strong C-C bonds (Adamczyk et al 2017, Bending and Read 1996, Kögel-Knabner 2002). Tannins are actively toxic towards microorganisms and function as a defense mechanism in the living plant but have also been documented to exhibit persistent effects beyond their presence in plants (Prigione et al 2018, Kögel-Knabner 2002). Their active toxicity stems from their unique ability to efficiently form complexation at their hydroxyl groups (Kilmister et al 2016) which may in some cases deform and harm microbial exteriors upon interaction (Prigione et al 2018). Commonly, tannins stabilize free organic matter in the soil, and their rare capacity to bind proteins, thus enzymes, is considered a significant factor in regulating decomposition of soil litter, in part due to catabolite suppression (Adamczyk et al 2017, Joanisse et al 2007, Bending and Read 1996). Under prevailing soil infertility, tannin production may increase to aid plant nutrient conservation (Bending and Read 1996).

Tannins can be divided into two groups: condensed and hydrolysable (Adamczyk et al 2017, Prigione et al 2018). As the name suggests, the latter can be decomposed by hydrolytic enzymes, which many microorganisms produce. Condensed tannins (CT) are more difficult to break down due to their random and complex structure. Few organisms have the capacity to efficiently break the chemical bonds as CT mostly lack the oxygen or nitrogen bridge required for hydrolysis (Prigione et al 2018). Organic nitrogen complexed with tannins thus often remain in this state for extensive periods of time.

Tannins contribute to up to 20 % of the plant dry weight (Adamczyk et al 2017) and are unevenly distributed throughout the plant physiology. In the overall terrestrial biomass, it is the third most abundant component after carbohydrates and lignin (Hernes & Hedges 2004), but certain plant soft tissues such as needles and fine roots are enriched in tannins.

To summarize, the molecular structure of nitrogen affects what microbial enzymatic reactions are triggered and which paths through the ecosystem nitrogen will take. Mineral nitrogen is readily available for uptake, and is often quickly biosynthesized. Organic nitrogen like proteins are susceptible for hydrolysis and can be catabolized by all organisms using hydrolases. Recalcitrant nitrogen, where organic nitrogen is in complexation with e.g. condensed tannins, often remain in the soil for extended periods of time, as only a few groups of microorganism are able to dismantle these complex materials.

1.2 Fungi

Fungi are a diverse group of heterotrophic eukaryotic microorganisms (Naranjo-Ortiz & Gabaldón 2019). They are distinct from plants, animals, and bacteria in terms of their biology and characteristics, and inhabit a wide spectrum of niches, yet our knowledge about them is limited. Fungi exhibit tremendous diversity, with over 100,000 known species and potentially millions more yet to be discovered. They can range from microscopic single-celled yeasts to large, complex multicellular organisms like filamentous "mushroom forming" fungi (Naranjo-Ortiz & Gabaldón 2019).

Many species of fungi form mycelium, the vegetative and often underground part of a fungus (Islam et al 2017). It consists of a network of thread-like structures called hyphae, which are the primary feeding and growing structures of the fungus. These hyphae are composed of nitrogen-rich compounds such as chitin and proteins (Islam et al 2017) and fungal growth occurs through apical extension by high internal pressure and though exocytosis (Hernández-González et al 2018, Grove & Bracker 1970). Through exocytosis, the fungus also secretes catabolic enzymes into its surroundings to depolymerize macromolecules in order to retrieve nutrients and carbohydrates. As opposed to members of most other kingdoms, there is no limit to how large some individual fungal mycelium can grow and how old they can become, was it not for external factors (Anderson et al 2018).

Fungi can be divided into three main categories of nutrient acquisition; biotrophic, necrotrophic and saprotrophic. Biotrophic fungi form mutualistic relationships with a host organism, often plants, by partially growing inside them and feeds on the living cells of their hosts in exchange for providing it with soil nutrients such as nitrogen (Behnie & Bidochka 2014). Necrotrophic fungi harm their host, possibly killing them, in order to acquire the nutrients needed for their survival (Solomon et al 2003). Saprotrophic fungi break down dead organic matter to meet their nutritional needs and serve as principal decomposers of organic material (Boddy & Hiscox 2016, Voříšková et al 2011).

To obtain nutrients tied to biotic matter, fungi excrete a varied array of enzymes into their surroundings. All known fungal species uses a series of hydrolytic enzymes (HE) to break down complex molecules into simpler and simpler components, until the components has reached a form which can be absorbed into the fungal body (chemistryexplained.com). Hydrolytic enzymes are highly specific and their products are thus very predictable, and they can be categorized into different catabolic functions such as cellulases, lipases and peptidases. Leucine aminopeptidase (LAP) is a peptidase which catalyzes the hydrolysis of proteins and peptides at the N terminus (Gu et al 1999), and is probably used by all fungi. Some fungi have, in addition to specialized hydrolytic enzymes, oxidative enzymes which allow these species to depolymerize structures that lack the nitrogen and oxygen bridges required for hydrolysis, hence breaking down stubborn organic material to salvage the otherwise scarce nitrogen resources.

Oxidative enzymes (OE) are unspecific and highly reactive enzymes often used by some fungi to degrade resilient organic matter and as defense against external attack. "Oxidative enzymes" is a broad term that encompasses a wide range of enzymes involved in biochemical reactions that either transfer electrons during oxidation-reduction (redox) reactions or promote the generation of reactive oxygen species (ROS) as part of their normal catalytic function. These enzymes free compounds including proteins and chitin from covalent complexes with e.g. lignin and tannins, whereafter hydrolytic enzymes gain access to further depolymerize the macromolecules required for mycelial growth (Voříšková et al 2011). Manganese peroxidases (MnP), along with other enzymes like lignin peroxidase (LiP) and laccase, contributes to the degradation of polymers such as lignin and tannins by catalyzing oxidative reactions. Specifically, MnP oxidizes free manganese ions (Mn^{2+}) in the soil into highly reactive radicals (Mn^{3+}). To regain a stable electron charge, these radicals strike and break the chemical bonds in recalcitrant organic matter in their surroundings, leading to its decomposition into smaller, often more manageable fragments, which can then be further depolymerized through hydrolysis. To recharge the MnP enzyme, the fungus oxidizes it again by excreting hydrogen peroxide (H_2O_2). Production of MnP is a one-time energy investment by the white-rot fungi, but the process of recharging it by hydrogen peroxidase production probably has a high and continuous energy cost (Shimizu et al 2005). However, this high energy cost is often outweighed by the competitive advantage gained from the subsequent capacity to retrieve vital and rare nutrients.

Enzyme production has been observed in some cases to be a response to the environment (Šnajdr et al 2010), Okal et al 2020). Oxidative enzyme (OE) production is regulated by several factors including catabolic by-products of e.g. tannins (Prigone et al 2018) and nutrient starvation (Kirk & Tien 1988). Limited N availability can trigger OE production (Janusz et al 2013, Rüttimann-Johnson et al 1994, Kirk & Tien 1988) and OE activity in forests soils have been found to decrease after fertilization in some cases (Jörgensen et al 2021, Okal et al 2020, Janssens et al 2010), but increase in others (Voříšková et al 2011, Janssens et al 2010), while yet in others no effect is reported (Kirk & Tien 1988). Cellulase activity is reportedly influenced by growth substrate and conditions, and to increase following nitrogen input (Jörgensen et al 2021, Okal et al 2020). This discrepancy of enzyme response between studies may be due to variations in initial and consequent community composition (Jörgensen et al 2021), this is supported by the varying effects on fungal biomass which has been shown to increase, decrease, or remain unaffected following fertilization of forest soils (Jörgensen et al 2021, Kirk & Tien 1988). It is still poorly understood if and how ligninolytic fungi adapts their enzymatic production to nitrogen availability which makes it an interesting case to study.

Saprotrophic basidiomycetes are essentially the only fungi capable of producing potent peroxidases (Floudas et al 2012). Some of these mushroom forming fungi are considered our main wood decomposers, and are thus key players in the global carbon cycle (Voříšková et al 2011), due to their efficiency in breaking down complex plant structures. They occupy varying habitats and utilize diverse nutrient sources. Three well-known MnP producers are *Hypholoma fasciculare, Mycena epipterygia* and *Marasmius oreades. H. fasciculare* is a wood-associated and litter utilizing species with slow and nonselective lignin decaying abilities, observed to efficiently utilize mineral nitrogen (Voříšková et al 2011, Šnajdr et al 2010). *M. epipterygia* is a litter decomposing fungi with a recognized efficiency to break down needles (Boberg et al 2011). *M. oreades* commonly thrives on turfgrass, lawns or other grasslands, and is known to create ”fairy-rings” (Djajakirana et al 1996).

1.3 Purpose and issue/hypothesis

The aim of this paper is to investigate if and how saprotrophic basidiomycetes adapt their enzyme activity to N availability, and how it affects their biomass production. The fungi will be given high and low concentrations of readily available (inorganic), easily acquirable (organic) or recalcitrant (CT-complexed organic) nitrogen, and activity of manganese peroxidase (MnP) and Leucine aminopeptidase (henceforth referred to as peptidase) will be assayed.

We hypothesize that the fungi will regulate activity of both Mn peroxidase and peptidase in response to how easily acquirable N is. As inorganic ammonium (NH_4) is readily available for the fungus and requires neither MnP nor peptidase to attain, we expect no significant activity of either enzyme in mediums with NH_4 (Table 1). Organic nitrogen bound in proteins can be accessed through peptidase reactions and does not require oxidative enzymes, therefore we predict that fungi given organic nitrogen will have high peptidase activity and insignificant MnP activity. Organic nitrogen stabilized by condensed tannins requires oxidative enzymes to free the nitrogen from complexation, whereafter peptidase have access to depolymerize the structures further. Thus we hypothesize that fungi given CT-complexed organic nitrogen will have a high MnP activity, with a possible decrease over time in conjunction with increased peptidase activity. If N starvation can induce MnP production, we hypothesize that the replicates under complete nitrogen starvation (no added N) is expected to trigger high MnP activity. Biomass is expected to be highest in the treatments with high concentrations of inorganic nitrogen, as the fungi spends insignificant energy on enzyme activity. Given organic nitrogen, biomass yield is expected to be slightly lower than from mineral nitrogen mediums, as some energy is spent on peptidases. If MnP maintenance is energy costly, we expect fungi provided with recalcitrant organic matter to have lower biomass yield than when the fungi is provided with mineral or organic nitrogen. Lowest biomass yield is expected from replicates under complete nitrogen starvation, as these will have nothing to biosynthesize.

Table 1. Hypothesised response from fungi to varying mediums. Expectation of trends of enzyme activities over three time points in response to different mediums. Expectation of relative biomass gain compared to replicates under nitrogen starvation.

Response	Inorganic High	Inorganic Low	Organic High	Organic Low	CT High	CT Low	Nitrogen Starvation
MnP	↘↘↘	↘↘↘	↘↘↘	↘↘↘	↗↗↘	↗↗↘	↗↗↘
Peptidase	↘↘↘	↘↘↘	↗↗↗	↗↗↗	↘↘↗	↘↘↗	↗↗↘
Biomass	++++	+++	+++	++	++	+	

2. Material and method

Laboratory research on three species of saprotrophic basidiomycetes was carried out under sterile conditions at the department of Forest Mycology and Plant Pathology at the Swedish University of Agricultural Sciences (SLU) campus Ultuna in Uppsala, Sweden. *Hypholoma fasciculare* JB 13 Uppsala (henceforth referred to as Hypholoma)*, Mycena epipterygia* MUCL 047611 (henceforth referred to as Mycena) and *Marasmius oreades* MUCL 028591 (henceforth referred to as Marasmius) were all obtained from the culture collection at the department of Forest Mycology and Plant Pathology at the Swedish University of Agricultural Sciences (SLU) campus Ultuna in Uppsala, Sweden. The three species were maintained in a dark room at 20°C, grown on malt agar. Purified condensed tannins extracted from fine pine roots (2022) were kindly provided by Bartosz Adamczyk at the University of Helsinki.

Malt plugs from three species of saprotrophic basidiomycetes (*Hypholoma fasciculare, Mycena epipterygia, Marasmius oreades*) were placed on a layer of 5 mm glass beads in Petri dishes with 10 mL of modified liquid MMN medium (Rüttimann-Johnson et al 1994, Droce et al 2013) to allow the fungi to gain biomass before the experiment. After five weeks, the liquid from each Petri dish was replaced with mediums of varying N content. From this, enzyme assays were sampled weekly for three weeks and enzyme activities analyzed fluorometrically and colorimetrically to determine hydrolytic and oxidative enzyme activity respectively. Biomass was collected at the end of the laboratory experiment. Data was handled in Microsoft Excel (version 16.78) and analyzed statistically in RStudio Version 2023.06.1+524 (2023.06.1+524).

2.1 Laboratory work

2.1.1 Medium

Two stages of liquid medium cultivation were performed. Mediums were chosen to be liquid to facilitate enzyme sampling (Droce et al 2013). For the first stage, liquid MMN medium was prepared (Bending and Read 1996). Recipe was modified by exchanging malt extract with glucose as the carbon source, by adding nitrogen as ammonium tartrate, and agar was excluded to maintain liquid state. This modified MMN contained all essential nutrients fungi requires to allow biomass production.

For the second stage, fungi were given high (12 mM) and low (1.2 mM) nitrogen concentrations (Li et al 1995) in various forms. These were all made by first preparing a liquid base solution containing all essential nutrients but nitrogen (except in thiamine), and then adding nitrogen in the concentration and form required for each treatment.

In a pre-experiment trial, only 8 mL of the added 10 mL liquid could be retrieved from the layer of glass beads in a Petri dish, as the remaining 2 mL were bound tightly to the glass bead surfaces. The concentration of N in the prepared mediums for the main experiment (second stage) were thus adjusted to 15 mM and 1.5 mM, and 8 mL was added to the already present 2 mL (nitrogen free medium), to attain 10 mL 12 mM and 1.2 mM respectively.

Base solution

The second stage's liquid base medium contained the following: 0.15 g/L $MgSO_4$, 0.05 g/L $CaCl_2$, 0.05 g/L NaCl, 1.2 % $FeCl_3$, 0.1 % thiamine, 0.5 g/L KH_2PO_4, 295 µM $MnSO_4$, 5 g glucose, and trace elements modified from Vogels minimal medium; 5 g citric acid * 1 H_2O, 5 g $ZnSO_4$ * 7 H_2O, 0.25 g $CuSO_4$ * 5 H_2O, 0.05 g H_3BO_3 anhydrous, 0.05 g Na_2MoO_4 * 2 H_2O (diluted 50 * 200). pH was adjusted to 4.5 with 1 M NaOH. No nitrogen was added (except though thiamine). The medium was sterilized in an autoclave. Henceforth this solution will be referred to as No N.

Inorganic nitrogen

Ammonium tartrate ($(NH_4)_2C_4H_4O_6$) weighing 830 mg (MW 184.15 Da) was mixed with 50 mL No N medium and sterilized through a 0.22 µm filter syringe into a bottle (Guillén et al 1994). Medium containing no nitrogen (No N) was then added to the bottle to reach 300 mL. The process was repeated with 83 mg ammonium tartrate. Henceforth referred to as NH_4.

Organic nitrogen

Bovine serum albumin fraction V (BSA) (N-content 16 % (Sigma-Aldrich)) (Šnajdr 2010) weighing 151 and 15.1 mg was added to 10 mL distilled water respectively, sterilized through a 0.22 µm filter syringe (Guillén et al 1994), and added to 90 mL No N medium. Henceforth referred to as BSA.

CT complexed organic

Bovine albumin fraction V (BSA) weighing 176.5 mg was added to 71 mL 0.2 M acetic buffer (pH 4.9) and sterilized through a 0.22 µm filter syringe (Adamczyk 2023 personal communication). CT weighing 151 g was mixed with 100 mL autoclaved ultrapure water, and sterilized through 0.2 µm filter syringe. Equal volumes CT and BSA solution were mixed in weighted 15 mL falcon tubes, shaken on orbital shaker (200rpm) in horizontal position for one hour and centrifuged at 2500g for 5 minutes. The supernatant was gently removed, 5 mL water added, mixed and centrifuged – this was repeated three times to remove unbound CT and BSA. CT:BSA complexes were freeze dried and weighted. To find the molecular weight of CT:BSA complexes, the mass was measured and the molecular weight calculated assuming 85 % had formed complexes. Then, 211 mg CT:BSA was added to 100 mL No N medium. Henceforth referred to as CT.

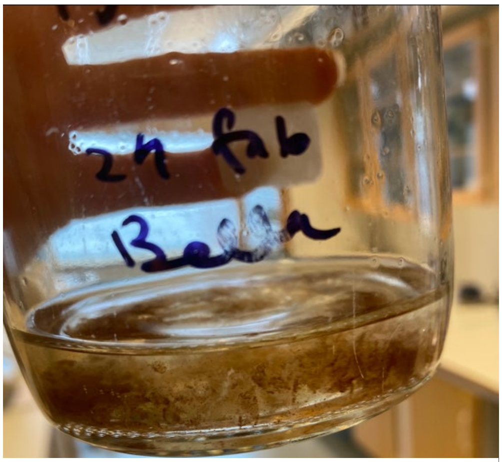


Figure 1. Medium containing suspension of CT:protein complexes.

In all, seven mediums were prepared; two solutions containing inorganic nitrogen (high/low) (NH_4), two solutions containing organic nitrogen (high/low) (BSA), two suspensions containing organic nitrogen complexed with condensed tannins (high/low) (CT) and one solution with no added nitrogen (No N).

2.1.2 Cultivation

In preparation, the three fungal strains were grown on malt agar in a dark room at 20°C for 10 days. From this, 5 mm plugs were translocated to a Petri dish containing 10 mL liquid modified MMN (first stage) and one layer of 5 mm glass beads to support fungal hyphae (Droce et al 2013) (Figure 2). For each fungi, 35 replicates were prepared. The fungi were then grown in a dark room at 20°C for five weeks.

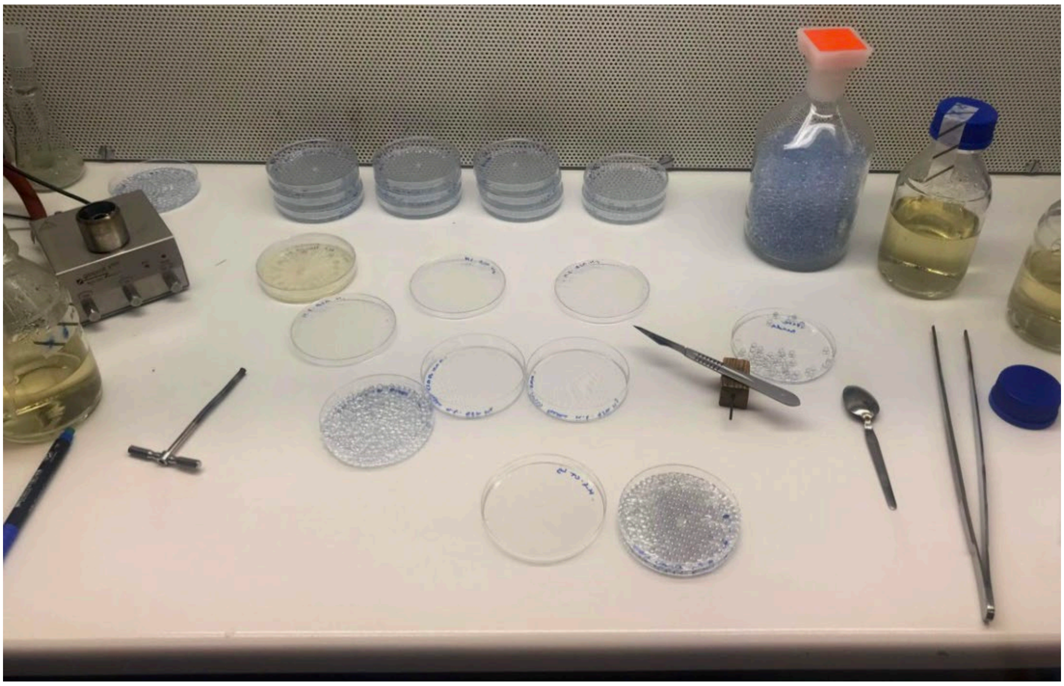


Figure 2. Process of placing fungi on a layer of glass beads. First stage of liquid medium, where all replicates were given modified MMN containing plenty of nitrogen.

After five weeks, as much liquid MMN as possible (5-7 mL) was carefully removed using a PIPETBOY, and the mycelium was rinsed three times with medium containing no nitrogen (No N). Mediums with high and low concentration of NH_4, BSA and CT, or with No N, was added to five Petri dishes each, carefully to minimize disturbance of the mycelia that extended across the glass beads (second stage) and stored in a dark room at 20°C. Enzyme sampling started after one week.

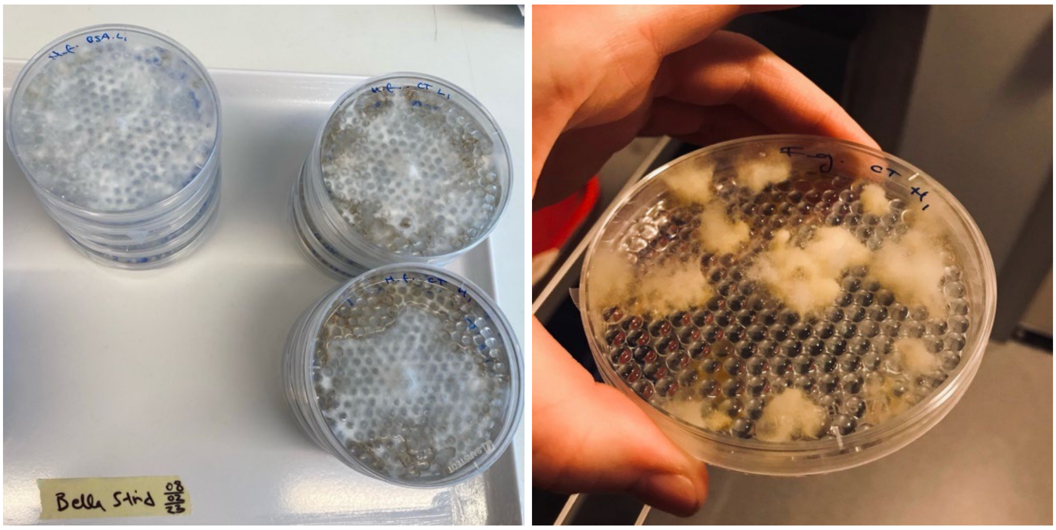


Figure 3. Mycelial growth of Hypholoma fasciculare (left) and the Ascomycete Fusarium graminearum (right). F. graminearum was excluded from the experiment due to time limitations.

2.1.3 Enzyme assays

From each Petri dish, four samples of 100 µL and four samples of 80 µL fungal supernatant was collected with a pipette weekly for three weeks (Šnajdr et al 2010). The four 80 µL samples were incubated at 90°C for 15 minutes to inhibit all enzyme activity and were later used as negative controls. Samples were stored in -20°C for a few weeks before analysis. Analyses were conducted as described by Kyaschenko et al (2017).

MnP analysis

MnP activity was assessed colorimetrically in a SpectraMax® Plus^{384} Absorbance Microplate Reader (Molecular Devices) (Figure 4) through a reaction involving 3-methyl-2-benzothiazolinone hydrazone (MBTH, 1 mM) and 3-(dimethylamino) benzoic acid (DMAB, 50 mM), carried out in the presence of both Mn^{2+} ($MnSO_4$ * H_2O, 1 mM) and H_2O_2 (Ehlers & Rose 2005). All reagents were dissolved in sodium acetate buffer (acid base), and working solutions contained 2.5 mL 100 mM sodium lactate buffer, 2.5 mL 100 mM sodium succinate buffer, 0.5 mL DMAB solution, 0.5 mL MBTH suspension and 1 mL $MnSO_4$ solution. To account for any potential interference from laccase, which can also utilize MBTH and DMAB as substrates, parallel analyses were conducted with adjusted ingredients. Specifically, manganese sulphate was substituted by an equimolar amount of ethylenediaminetetraacetic acid (Na_2-EDTA * H_2O, or EDTA, 1 mM) was introduced into the reaction mixture, to chelate manganese ions and prevent Mn-dependent oxidation. Into each well in a clear, flatbottomed, 96-welled PCR plate, each of the following was added; 141 µL of solution containing $MnSO_4$ or solution containing EDTA, 40 µL acid base solution and 10 µL of sample. The reaction was initiated by adding 10 µL 5 mM H_2O_2. The plate was read at 590 nm at 25°C, every 2 minutes for 30 minutes.

Figure 4. SpectraMax® Plus^{384} Absorbance Microplate Reader used for analysis of Mn peroxidase activity in the foreground. LS50B Luminescence Spectrometer used for analysis of peptidase activity in the background.

Peptidases analysis

Activity of peptidase was determined through single- molecule fluorescence spectroscopy. Into each well in a black, flatbottomed, 96-welled PCR plate, each of the following was added; 190 µL acid base solution, 50 µL Leucine aminopeptidase (LAP) and 10 µL sample.

To stop the reaction 10 µL 0.5 M NaOH was added. For each set of samples, two plates were prepared; one was immediately stopped by addition of NaOH while the other was incubated in darkness at room temperature for 45 minutes until stopped by NaOH. Ten minutes after NaOH addition, the plate was read by the LS50B Luminescence Spectrometer (PerkinElmer instruments) (Figure 5) with the following settings: excitation wavelength 365 nm, excitation slit 2.5, emission wavelength 450 nm, emission slit 2.5, emission filter 390 cut off, one reading per cycle, reading time 1 second per well. As analysis was conducted in order to determine differences between treatments and not the absolute activity, no control of autofluorescence was done.

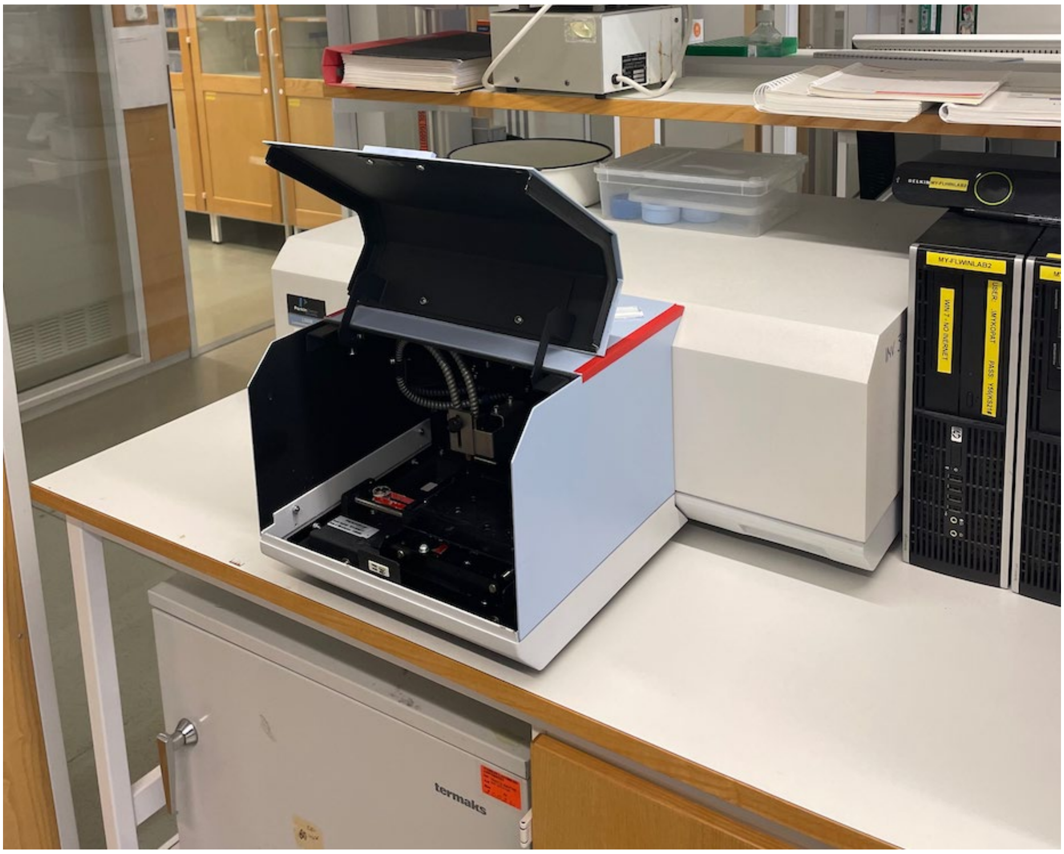


Figure 5. LS50B Luminescence Spectrometer used for analysis of peptidase activity. Oven used for drying biomass pictured in the bottom.

2.1.4 Biomass

To measure fungal biomass, contents of the Petri dish was emptied into a 1 L beaker. Water was added and the content was vigorously stirred to separate hyphae from the glass beads. The liquid was then carefully poured over a pre-weighed 15 cm filter paper. This was repeated 5-8 times until all biomass was assumed to be extracted. The filters were folded and oven-dried at least 48 hours before measuring weight. Net biomass was calculated as the difference between initial paper weight and weight of paper with biomass.

2.2 Data processing and analysis

Data was handled in Microsoft Excel (version 16.78) and analyzed statistically in RStudio version 2023.06.1+524 (2023.06.1+524).

2.2.1 Microsoft Excel

For enzyme activities, slopes of absorbance over time was calculated. Activities were converted into rate per minute by dividing the measured activity by the measurement frequency for each assay (MnP by 2 and peptidase by 45). Determination of MnP activity was achieved by subtracting the Mn-independent activity from the total peroxidase activity observed in the reaction. Determination of peptidase activity was achieved by subtracting fluorescence of the reaction stopped immediately from the fluorescence of the reaction stopped after 45 minutes. All negative values for both Mn peroxidase and peptidase were considered to be activity below detection limit and thus treated as zero. All values from mediums with NH_4, BSA protein and CT:protein complexes were divided by the mean activity of mediums with No N per treatment and time point.

Biomass responses to varying nitrogen sources was estimated as follows: mean biomass of the replicates grown under nitrogen starvation (No N) was calculated and then subtracted from all other biomass measurements of that fungus (grown in NH_4, protein or CT:protein complexes). As condensed tannins were challenging to remove from the biomass during laboratory work, calculations were made to estimate the mass of added CT:BSA complexes to each replicate, which was later subtracted from the total measured biomass. Estimated weight of CT:BSA complexes added to each Petri dish was calculated as follows (see chapter 2.1.2 for medium recipe):

$$151\, mg\ BSA \times\ 1.40\ = 211.4\, mg\ CT\colon BSA$$
$$211.4\, mg\ CT\colon BSA\ \times\ 0.008\, L = 1.69\, mg\ CT\colon BSA$$

Treatments with high and low recalcitrant nitrogen concentrations had received 1.69 mg and 0.169 mg CT-protein complexes respectively. Thus, these values were subtracted from respective measured biomass.

All graphs were prepared in Microsoft Excel.

2.2.2 RStudio

In RStudio, the data underwent statistical analysis using linear models, Repeated Measures ANOVA, and Tukey HSD. Packages used in RStudio was lme4, readr, dplyr and ggplot2. Functions used for analysis were *lm* (linear model), *anova*, *aov* and *TukeyHSD*. The statistical linear model, a framework expressing variable relationships through linear equations (Rushing et al., 2014), involves fitting a line to observed data for prediction and inference. Repeated Measures ANOVA, designed for experiments with repeated measurements on the same subjects, assesses within-subject variations over time or conditions. Terms like degrees of freedom (df), sum of squares (sumsq), and mean square (meansq) assess variability and group differences. Degrees of freedom represent values free to vary, sum of squares quantifies total variability, and mean squares reveal average variability. The resulting F-statistic and p-value guide ANOVA interpretation, indicating significant group differences (significance $p < 0.05$, trend towards significance $0.05 < p < 0.1$). To identify which specific pairs of groups differed significantly from each other, the post-hoc test Tukey multiple comparisons of means, 95 % family-wise confidence of means was performed. TukeyHSD (Tukey's Honestly Significant Difference) is a post-hoc test often used in analysis of variance (ANOVA) to compare multiple group means and determine which specific pairs of groups differ significantly from each other. This test helps identify where significant differences exist when you have conducted an ANOVA and found that there are differences among groups, but you want to pinpoint which groups are different from each other. The TukeyHSD test provides adjusted p-values to make these pairwise comparisons. Tukey test cannot however include the random effect ID, hence significant changes in individual replicates are not accounted for. Furthermore, significant difference in e.g. medium over time (medium:time interaction) excludes the factor concentration when ID is not included.

The response variable enzyme activity (relative to the No N treatment) was tested against factors medium (NH_4, BSA, CT), concentration (high, low) and time (first, second third) individually and in interaction, with the random effect of individual replicates (ID). As the medium with no nitrogen at all (No N) did not have a factor of concentration (high/low), it could not be included in the analysis together with NH_4, BSA protein and CT:protein complexes. To address this, an analysis was conducted on solely NH_4 (readily available N) and No N, where the concentration of No N was set to “none”. The data of NH_4 in this dataset was not divided by values of mean No N, but kept as raw values. In this analysis, the factor “medium” was excluded and thus run only on factors “concentration” (high, low, none) and “time”. This dataset is referred to as N control. As No N was not included in the main statistical analysis, these data points are not presented in the following graphs.

The response variable biomass (relative to the No N treatment) was tested against factors medium (NH_4, BSA, CT) and concentration (high, low) individually and in interaction. As for enzyme activity, additional analysis of biomass in No N medium and NH_4 medium was conducted, here also referred to as N control. Graphs show results raw data measurements from all seven mediums, including No N.

For all analyses, only pairwise comparisons of (trending towards) significant differences which were deemed relevant are presented in text and in graphs. All (trending towards) significant differences ($p < 0.1$) are found marked with a red dot in Appendix 1 and Appendix 2.

3. Results

3.1 Enzyme Activity

3.1.1 *Hypholoma fasciculare*

MnP activity

Initial analysis of N control showed no significance (Table 2).

Table 2. Analysis of Variance of MnP activity of Hypholoma in NH_4 and No N medium across time and concentration.

term	df	sumsq	meansq	statistic	p.value
Time	2	5,1E-11	2,5E-11	0,173	0,842
N availability	2	1,5E-10	7,3E-11	0,498	0,612
Time: N availability	4	1,0E-09	2,6E-10	1,759	0,157
Residuals	39	5,7E-09	1,5E-10		

Mean MnP activity per minute in No N medium at each time point was $2.9 * 10^{-6}$, $4.9 * 10^{-6}$ and $4.5 * 10^{-6}$ respectively, by which all other data points at the respective time point was divided.

Linear model of NH_4, BSA and CT showed significant difference between various interactions, see Figure 18 in Appendix 1. Analysis of variance (ANOVA) showed significant effect of time and of the interaction medium, concentration, and time (Table 3, Figure 6, Table 2).

Table 3. Analysis of Variance of MnP activity of Hypholoma across mediums, time and concentration.

term	df	sumsq	meansq	statistic	p.value
Medium	2	4,20	2,10	4,94	**0,010**
Time	2	4,32	2,16	5,08	**0,009**
Concentration	1	0,22	0,22	0,52	0,472
Medium:Time	4	3,43	0,86	2,01	0,102
Medium:Concentration	2	0,41	0,21	0,49	0,616
Time:Concentration	2	0,95	0,48	1,12	0,333
Medium:Time:Concentration	4	5,75	1,44	3,38	**0,014**

Residuals	72	30,64	0,43

Tukey test showed significant difference in MnP activity between mediums BSA and CT (p = 0.034), and NH_4 and CT (p = 0.015), where MnP activity in CT was lower than in both others, see Figure 20 in Appendix 1. Between first and third time point (p = 0.006) there was a significant decrease in MnP activity. Overall, no significance effect of nitrogen concentration on MnP activity was seen. MnP activity in BSA medium was significantly lower during the last time point than the first (p = 0.012). MnP activity in high concentrations of BSA decreased slightly between first and second time point (p = 0.09). No other mediums showed significant change over time. Activity decreased in BSA medium between first and second time point (p = 0.0012).

Variation between replicates was greatest when Hypholoma was given high concentrations of NH_4, and lowest when given low concentrations of CT. All mediums generated higher variations of MnP activity than their low concentration counterparts.

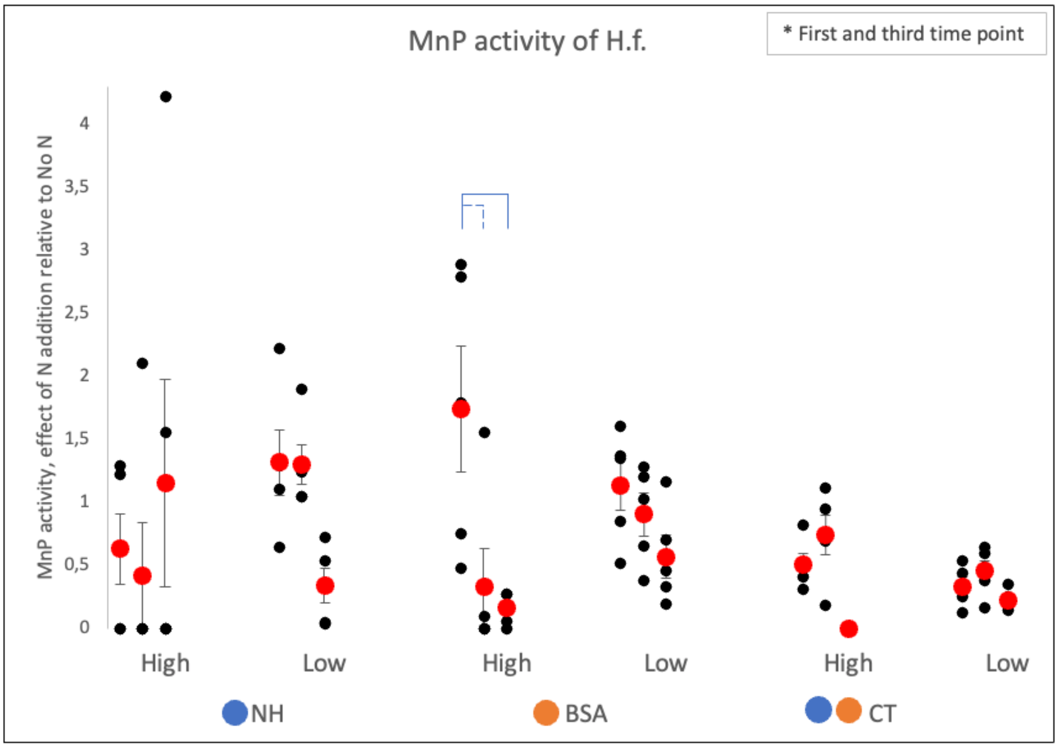


Figure 6. MnP activity of Hypholoma in six mediums. Data points are grouped per treatment (NH_4, BSA, CT), concentration (high (12 mM), low (1.2. mM)) and sampling time (first, second, third). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Dotted bars indicate trends towards significant difference between means. Solid circles of the same colour indicate significant difference between mediums. Text box lists significant differences not suitable for illustration.

Peptidase activity

Initial analysis of N control showed no significant differences (Table 4).

Table 4. Analysis of Variance of peptidase activity of Hypholoma in NH_4 and No N medium across time and concentration.

term	df	sumsq	meansq	statistic	p.value
Time	2	0,553	0,276	0,536	0,589
N availability	2	0,833	0,416	0,807	0,453
Time: N availability	4	1,754	0,439	0,850	0,502
Residuals	39	20,114	0,516		

Mean peptidase activity in No N medium at each time point was 0.22, 0.30 and 0.67 respectively, by which all other data points at the respective time point was divided.

Linear model of NH_4, BSA and CT showed no significant difference between interactions, see in Appendix 1. Analysis of variance (ANOVA) showed significant effect of time and concentration (Table 5, Figure 7).

Table 5. Analysis of Variance of peptidase activity of Hypholoma across mediums, time and concentration.

term	df	sumsq	meansq	statistic	p.value
Medium	2	0,006	0,003	0,868	0,424
Time	2	0,029	0,014	4,260	**0,018**
Concentration	1	0,014	0,014	4,326	**0,041**
Medium:Time	4	0,001	0,000	0,068	0,991
Medium:Concentration	2	0,004	0,002	0,659	0,520
Time:Concentration	2	0,009	0,004	1,314	0,275
Medium:Time:Concentration	4	0,002	0,000	0,139	0,967
Residuals	72	0,241	0,003		

Tukey test showed that peptidase activity increased significantly between the last two time points ($p = 0.013$). Overall, high nitrogen concentration had slightly higher peptidase activity than low nitrogen concentrations ($p = 0.04$), see Figure 23 in Appendix 1. No other significant differences were found.

Variation of peptidase activity was greater in high N concentrations than in low. During all time points and in all mediums, there was activity below detection limit.

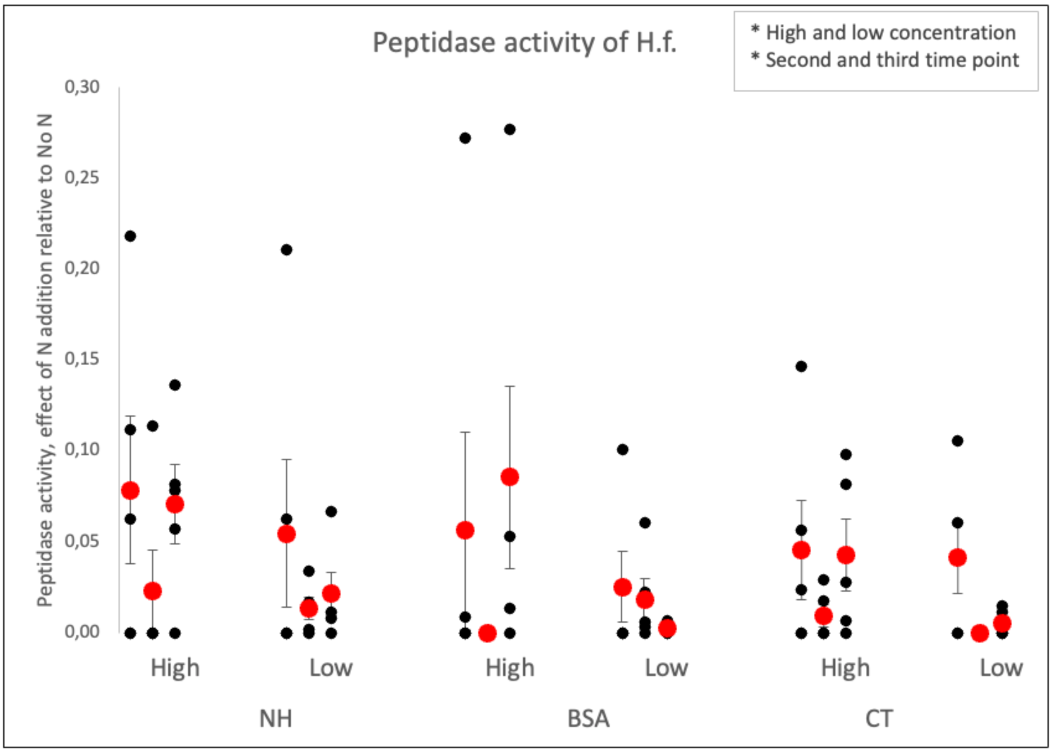


Figure 7. Peptidases activity of Hypholoma in six mediums. Data points are grouped per treatment (NH_4, BSA, CT), concentration (high (12 mM), low (1.2. mM)) and sampling time (first, second, third). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Text box lists significant differences not suitable for illustration.

Change in enzyme activities over time

Over all, the trend of Mn peroxidase and peptidase activity over time varies between all mediums and concentrations (Figure 8), with a slight similarity within CT treatments. MnP activity only increased between the first and last time point when the fungus was provided high concentration of readily available nitrogen (NH_4) and when provided no nitrogen at all (No N). MnP activity decreased in all other mediums between the first and last time point, including when nitrogen was complexed with condensed tannins. In CT:protein mediums, MnP peaked during the second week but was lower at the last time point than at initial sampling, whilst peptidase dipped during the second week. In treatments with high nitrogen concentration (NH_4, protein, CT:protein complexes), peptidase activity dipped at the second week. No N medium was the only medium where peptidase increased continuously over time.

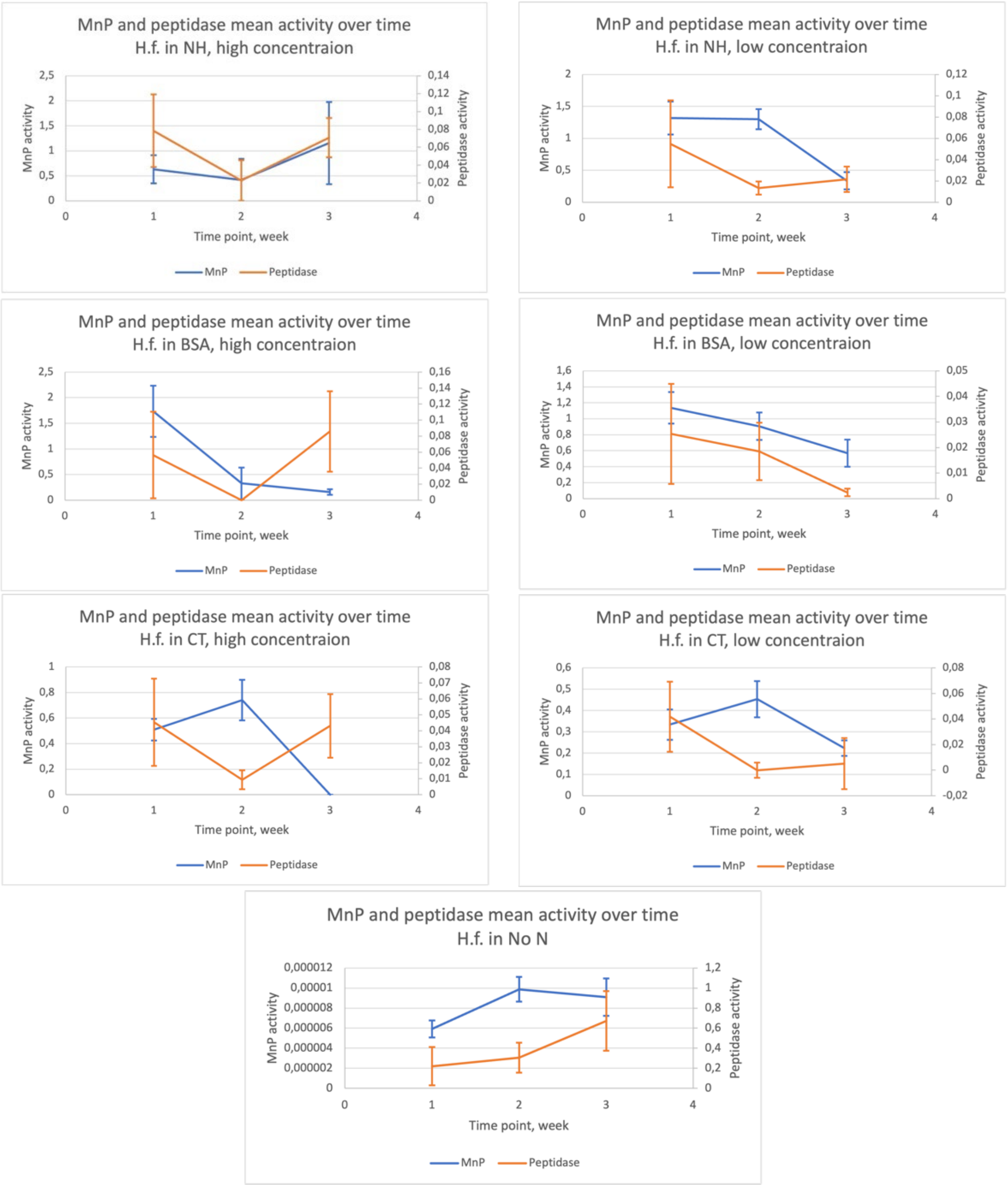


Figure 8. Mean MnP and peptidase activity of Hypholoma in different mediums over time (weeks). MnP activity on left y-axis, "MnP", blue line. Peptidase activity on right y-axis, orange line. Scales of y-axes differ within and between mediums.

3.1.2 *Mycena epipterygia*

MnP activity

Initial analysis of N control showed significant effect of concentration (p = 0.003) (Table 6). MnP activity in high nitrogen concentration was significantly greater than in both low nitrogen concentration (p = 0.008), and where no nitrogen was present (p = 0.005).

Table 6. Analysis of Variance of MnP activity of Mycena in NH_4 and No N medium across time and concentration.

term	df	sumsq	meansq	statistic	p.value
Time	2	7,68E-10	3,84E-10	0,619	0,544
N availability	2	8,58E-09	4,29E-09	6,910	**0,003**
Time: N availability	4	2,12E-09	5,31E-10	0,856	0,499
Residuals	39	2,42E-08	6,21E-10		

Mean MnP activity per minute in No N medium at each time point was $3.9 * 10^{-6}$, $4.8 * 10^{-6}$ and $8.3 * 10^{-6}$ respectively, by which all other data points at the respective time point was divided.

Linear model of NH_4, BSA and CT showed no significant difference between various interactions, see Figure 24 in Appendix 1. However, Analysis of variance (ANOVA) showed significant effect of nitrogen concentration (p = 0.03) (Table 7, Figure 9).

Table 7. Analysis of Variance of MnP activity of Mycena across mediums, time and concentration.

term	df	sumsq	meansq	statistic	p.value
Medium	2	10,437	5,219	0,633	0,534
Time	2	31,656	15,828	1,921	0,154
Concentration	1	39,189	39,189	4,755	**0,032**
Medium:Time	4	49,322	12,331	1,496	0,212
Medium:Concentration	2	14,967	7,483	0,908	0,408
Time:Concentration	2	1,035	0,517	0,063	0,939
Medium:Time:Concentration	4	9,249	2,312	0,281	0,890
Residuals	72	593,342	8,241		

Tukey test showed no significant effect of medium or of time. In high nitrogen, concentrations, MnP activity was much higher than in mediums with low nitrogen concentrations (p = 0.03), see Figure 26 in Appendix 1. No other significant effects were found.

Great variation of MnP production within replicates was observed for all mediums of Mycena In almost all cases (except NH_4 high third time point and NH_4 low first time point), there was some activity below detection limit. During the last two time points, all MnP activity in low concentrations of NH_4 were below detection limit.

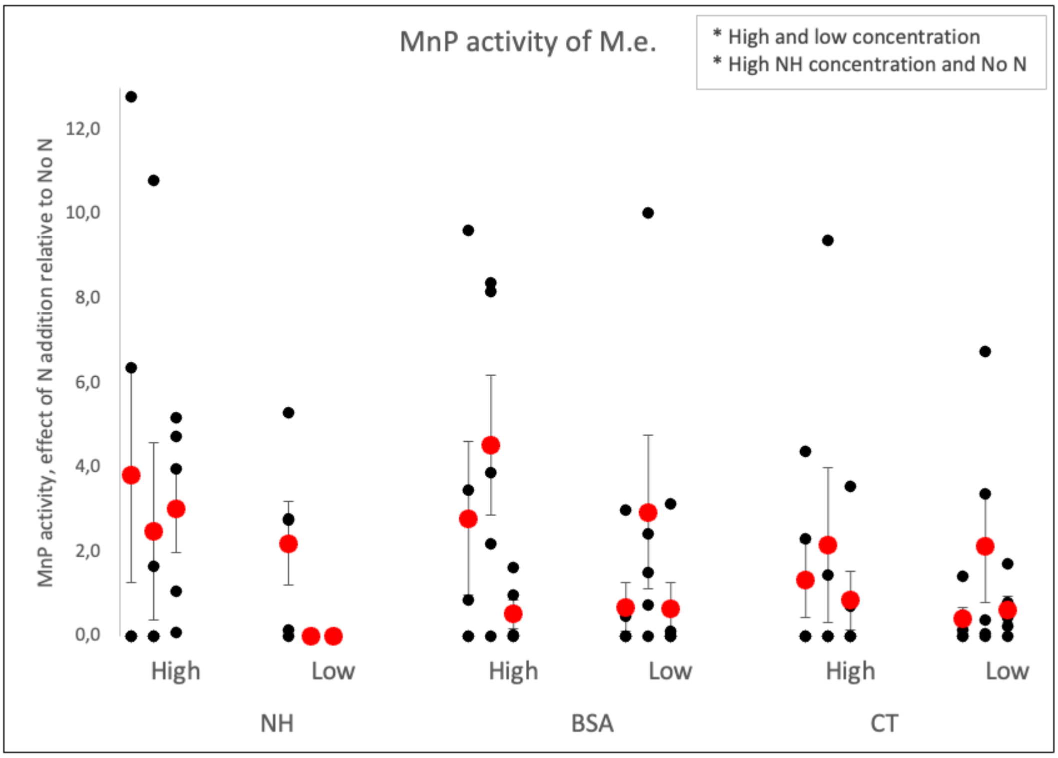


Figure 9. MnP activity of Mycena six mediums. Data points are grouped per treatment (NH_4, BSA, CT), concentration (high (12 mM), low (1.2. mM)) and sampling time (first, second, third). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Text box lists significant differences not suitable for illustration.

Peptidase activity

Initial analysis of N control showed no significant differences (Table 8).

Table 8. Analysis of Variance of peptidase activity of Mycena in NH_4 and No N medium across time and concentration.

term	df	sumsq	meansq	statistic	p.value
Time	2	1,195	0,597	0,396	0,676
N availability	2	1,985	0,992	0,658	0,524
Time: N availability	4	12,551	3,138	2,080	0,102
Residuals	39	58,843	1,509		

Mean peptidase activity in No N medium at each time point was 1.79, 0.83 and 0.32 respectively, by which all other data points at the respective time point was divided.

Linear model of NH_4, BSA and CT showed significant difference between high concentration in first time point and low concentration in third time point (p =

0.045), see in Appendix 1. Analysis of variance (ANOVA) showed significant effect of time, concentration, and interaction of time and concentration. Trend towards significance in interaction medium and time (Table 9, Figure 10).

Table 9. Analysis of Variance of peptidase activity of Mycena across mediums, time and concentration.

term	df	sumsq	meansq	statistic	p.value
Medium	2	0,004	0,002	1,328	0,271
Time	2	0,017	0,008	5,730	**0,005**
Concentration	1	0,013	0,013	8,791	**0,004**
Medium:Time	4	0,013	0,003	2,239	**0,073**
Medium:Concentration	2	0,002	0,001	0,562	0,573
Time:Concentration	2	0,026	0,013	9,049	**<0,001**
Medium:Time:Concentration	4	0,011	0,003	1,892	0,121
Residuals	72	0,105	0,001		

Tukey test showed significant increase of peptidase activity between first and third time point ($p < 0.001$) as well as a slight decrease between second and third time point ($p = 0.054$). In low nitrogen concentrations, peptidase activity was overall higher than in high nitrogen concentrations ($p = 0.04$), see Figure 28 in Appendix 1. In mediums with low nitrogen concentration, peptidase activity increased over time (first:third $p < 0.001$, second:third $p = 0.003$). Activity in low concentration of BSA increased significantly between first and second time point ($p = 0.012$) and between second and third time point ($p = 0.026$). In low concentration of NH_4, activity increased significantly between first and last time point ($p = 0.004$) and slightly between second and third time point ($p = 0.065$). During the last time point, peptidase activity was much higher in low concentrations of NH_4 than in high concentrations of NH_4 ($p = 0.003$). During the last time point, peptidase activity was highest in low concentration of NH_4 and BSA, both being significantly higher than that of all others, with the exception of BSA low and CT low which showed no significant difference in means.

Greatest variation of peptidase activity was found in low concentrations of NH_4 and of BSA, both during the third time point. In almost all cases (except NH_4 low third time point), there was some activity below detection limit.

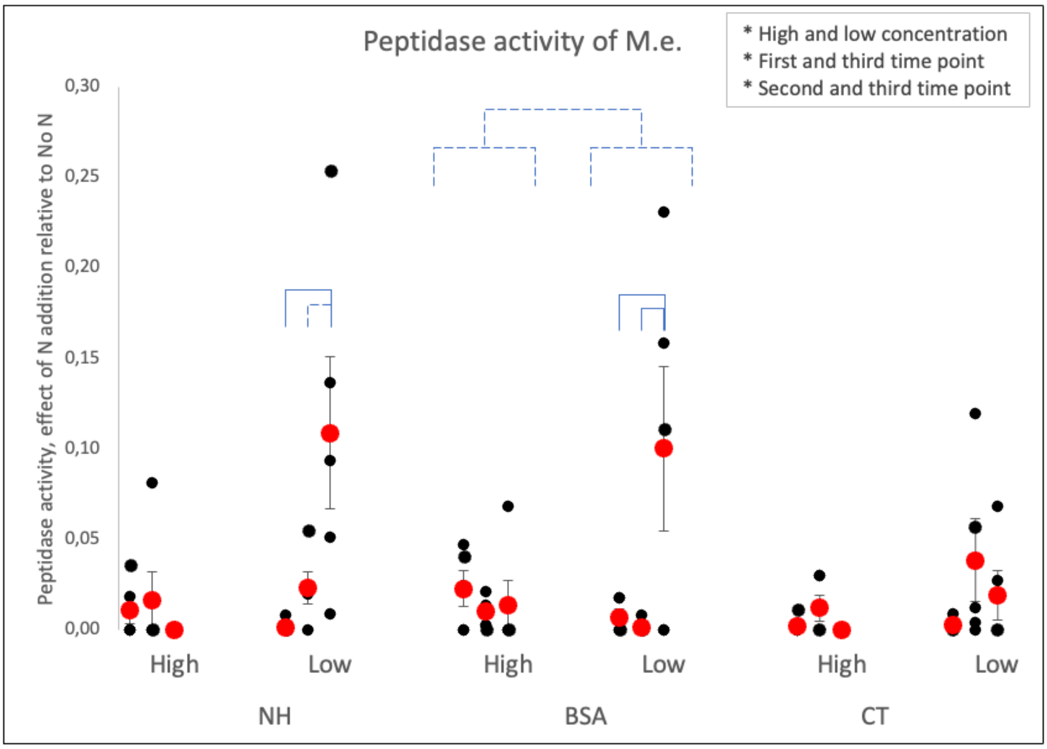
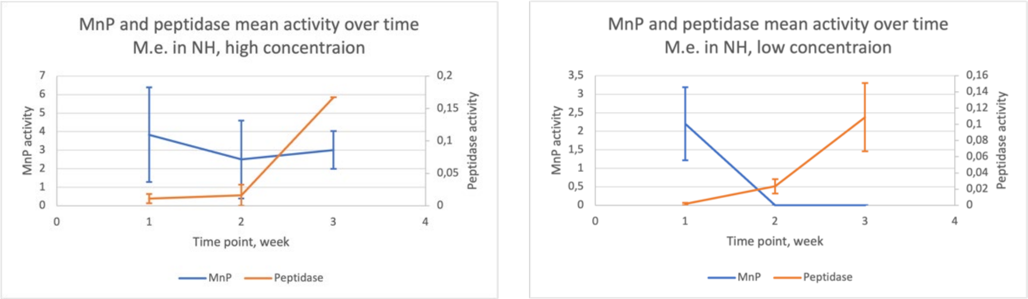


Figure 10. Peptidase activity of Mycena in six mediums. Data points are grouped per treatment (NH₄, BSA, CT), concentration (high (12 mM), low (1.2. mM)) and sampling time (first, second, third). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Solid bars indicate pairs with significant difference in means from Tukey Test. Dotted bars indicate trends towards significance. Text box lists significant differences not suitable for illustration.

Change in enzyme activities over time

Trend of enzyme activities over time often shared resemblance within the same medium at different concentrations (Figure 11). In NH_4 medium, Mn peroxidase activity was highest during the first week, while peptidase activity increased exponentially over time in both high and low concentration. In BSA medium, MnP activity peaks at two weeks, while peptidase slightly decreases from first to second week, to then increase greatly, in both high and low concentrations. In CT medium, both MnP and peptidase peak at the second week of sampling, in both high and low concentration. No N was the only medium where MnP activity continuously increased over time, while peptidase continuously decreased.



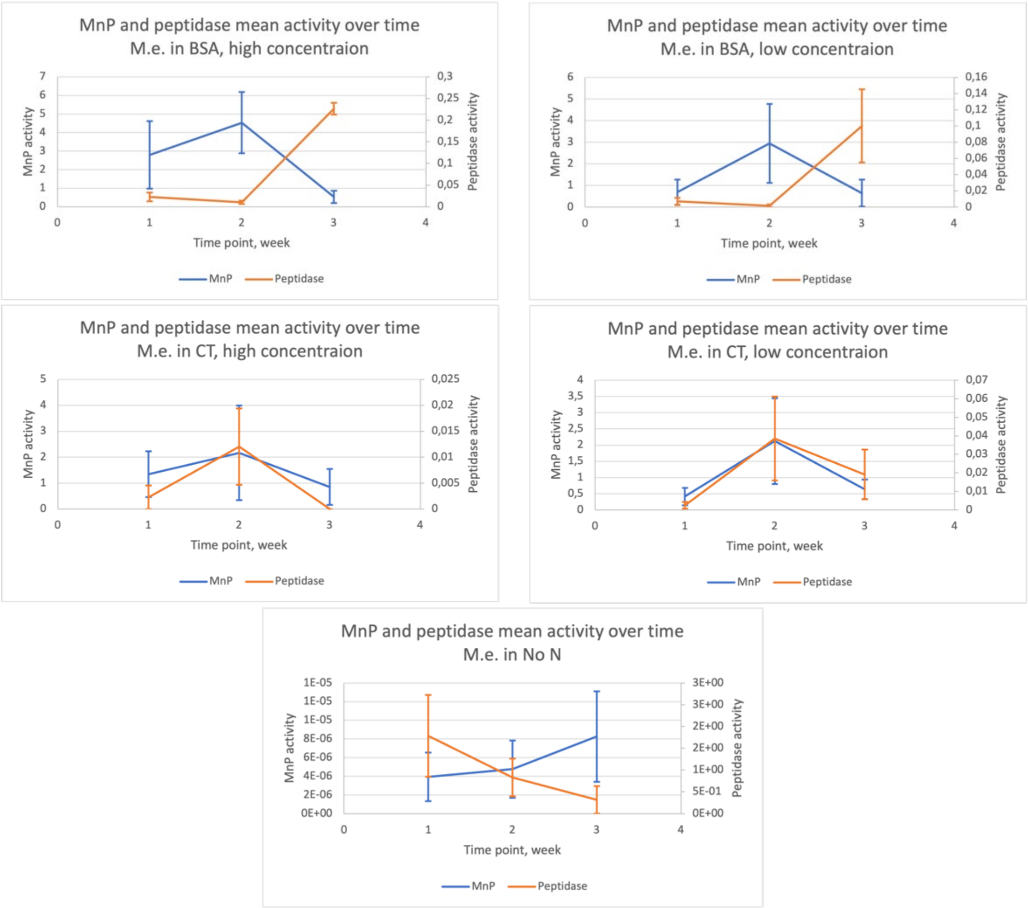


Figure 11. Mean MnP and peptidase activity of Mycena in different mediums over time (weeks). MnP activity on left y-axis, "MnP", blue line. Peptidase activity on right y-axis, orange line. Scales of y-axes differ within and between mediums.

3.1.3 *Marasmius oreades*

MnP activity

Initial analysis of N control showed significant effect of concentration (p = 0.039) (Table 10) and Tukey showed higher MnP activity in high concentrations of nitrogen than in in low concentrations (p = 0.04). There was no significant difference between No N and the others.

Table 10. Analysis of Variance of MnP activity of Marasmius in NH_4 and No N medium across time and concentration.

term	df	sumsq	meansq	statistic	p.value
Time	2	1,5E-12	7,3E-13	3,5E-01	0,705
N availability	2	1,5E-11	7,3E-12	3,5E+00	**0,039**
Time: N availability	4	7,7E-12	1,9E-12	9,2E-01	0,460
Residuals	36	7,4E-11	2,1E-12		

Mean MnP activity per minute in No N medium at each time point was $5.3 * 10^{-7}$, $9.4 * 10^{-7}$ and $8.4 * 10^{-7}$ respectively, by which all other data points at the respective time point was divided.

Linear model of NH_4, BSA and CT showed no significant difference between various interactions, see Figure 24 in Appendix 1. Analysis of variance (ANOVA) showed significant effect of time and of interaction medium and concentration (Table 11, Figure 12).

Table 11. Analysis of Variance of MnP activity of Marasmius across mediums, time and concentration.

term	df	sumsq	meansq	statistic	p.value
Medium	2	1,171	0,585	1,112	0,334
Time	2	4,306	2,153	4,090	**0,021**
Concentration	1	0,607	0,607	1,153	0,286
Medium:Time	4	1,198	0,300	0,569	0,686
Medium:Concentration	2	4,940	2,470	4,692	**0,012**
Time:Concentration	2	1,078	0,539	1,024	0,364
Medium:Time:Concentration	4	1,648	0,412	0,783	0,540
Residuals	72	37,899	0,526		

Tukey test showed no significant effect of medium or of concentration, but a significant decrease of MnP activity between first and second time point ($p = 0.015$), see Figure 32 in Appendix 1. In high NH_4 concentration, MnP activity was much higher than in low NH_4 concentrations ($p = 0.03$), and slightly higher than in high concentrations of CT ($p = 0.055$).

Variation of MnP activity was high in all mediums.

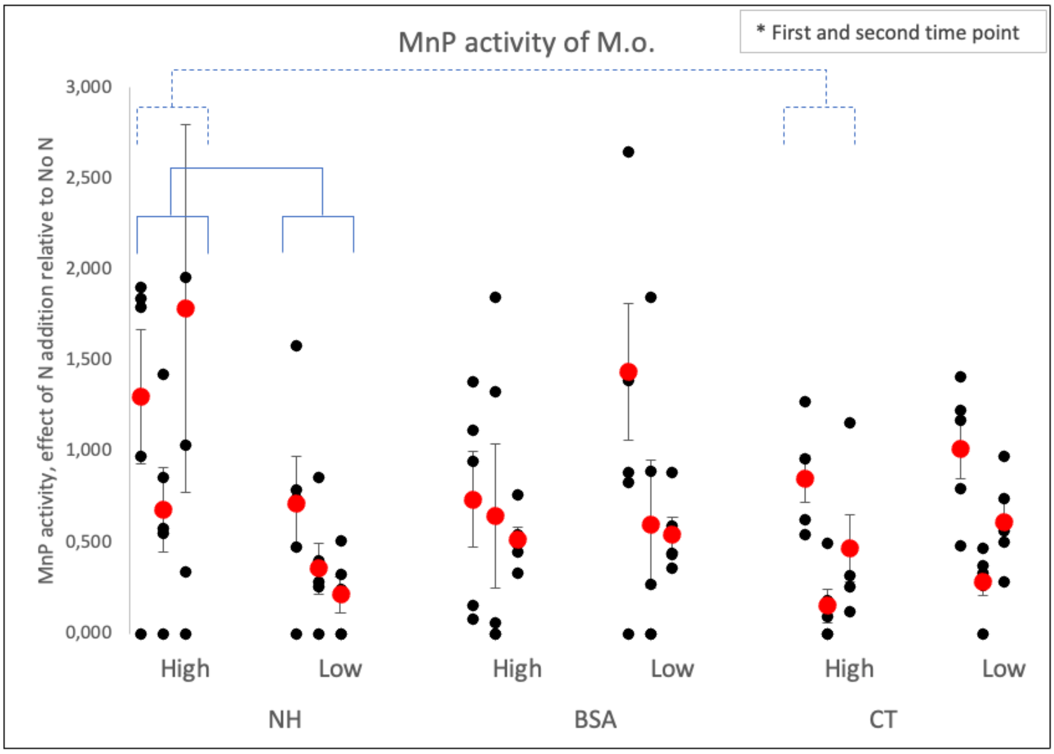


Figure 12. MnP activity of Marasmius in six mediums. Data points are grouped per treatment (NH_4, BSA, CT), concentration (high (12 mM), low (1.2. mM)) and sampling time (first, second, third). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Solid bars indicate pairs with significant difference in means from Tukey Test. Dotted bars indicate trends towards significance. Text box lists significant differences not suitable for illustration.

Peptidase activity

Initial analysis of N control showed no significant differences (Table 12).

Table 12. Analysis of Variance of peptidase activity of Marasmius in NH_4 and No N medium across time and concentration.

term	df	sumsq	meansq	statistic	p.value
Time	2	0,202	0,101	0,727	0,490
N availability	2	0,354	0,177	1,277	0,291
Time: N availability	4	0,667	0,167	1,203	0,326
Residuals	36	4,989	0,139		

Mean peptidase activity in No N medium at each time point was 0.36, 0.028 and 0.26 respectively, by which all other data points at the respective time point was divided.

Linear model of NH_4, BSA and CT showed no significant difference, see Figure 33 in Appendix 1. Analysis of variance (ANOVA) showed significant effect of time, medium, and interaction of time and medium (Table 13, Figure 13).

Table 13. Analysis of Variance of peptidase activity of Marasmius across mediums, time and concentration.

term	df	sumsq	meansq	statistic	p.value
Medium	2	0,141	0,070	3,266	**0,044**
Time	2	0,310	0,155	7,208	**0,001**
Concentration	1	0,052	0,052	2,437	0,123
Medium:Time	4	0,232	0,058	2,693	**0,038**
Medium:Concentration	2	0,062	0,031	1,441	0,243
Time:Concentration	2	0,055	0,027	1,272	0,287
Medium:Time:Concentration	4	0,149	0,037	1,725	0,154
Residuals	72	1,551	0,022		

Tukey test showed significant increase in peptidase activity between first and second time point (p = 0.0025), and significant decrease between second and third time point (p = 0.008), see Figure 35 in Appendix 1. Peptidase activity was slightly lower in CT mediums than in BSA mediums (p = 0.9), and than in NH_4 mediums (p = 0.06). Mediums with NH_4 increased their peptides activity between first and second time point (p = 0.013), and decreased it between second and third time point (p = 0.008), especially when in high concentrations (p = 0.004 and p = 0.0025 respectively). At time point 2, peptidase activity was highest in NH_4 high, being sing higher than both NH_4 L, CT H and CT L (0.03, 0.0016, 0.0016 respectively).

Variation of peptidase was generally greatest during the second time point.

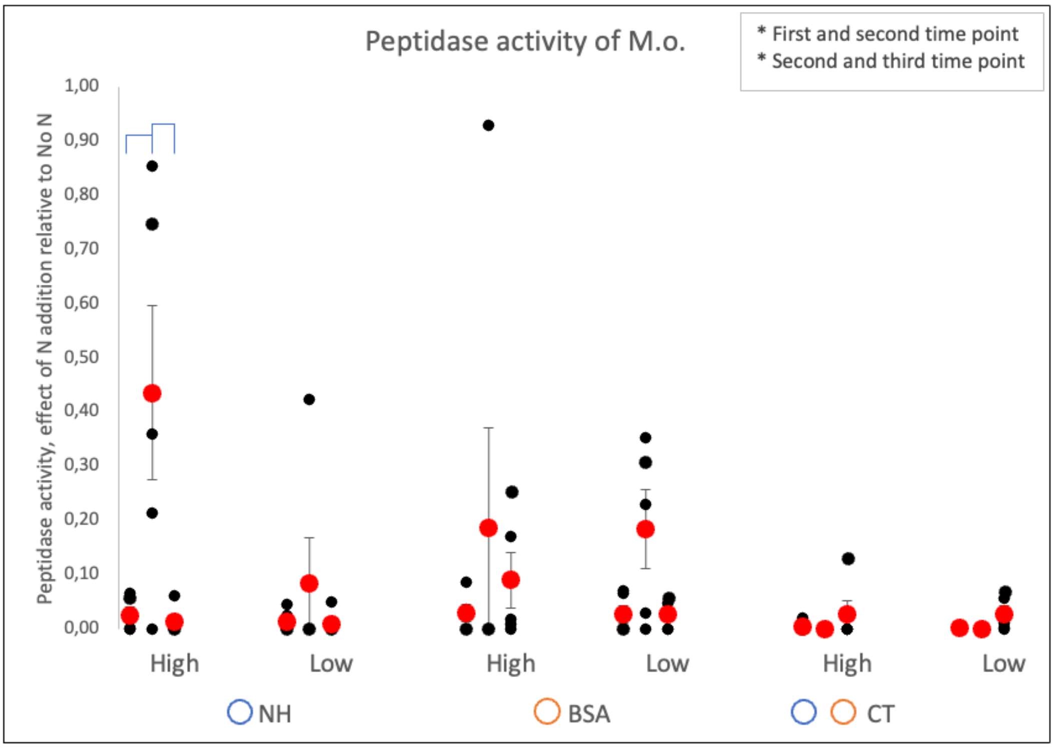
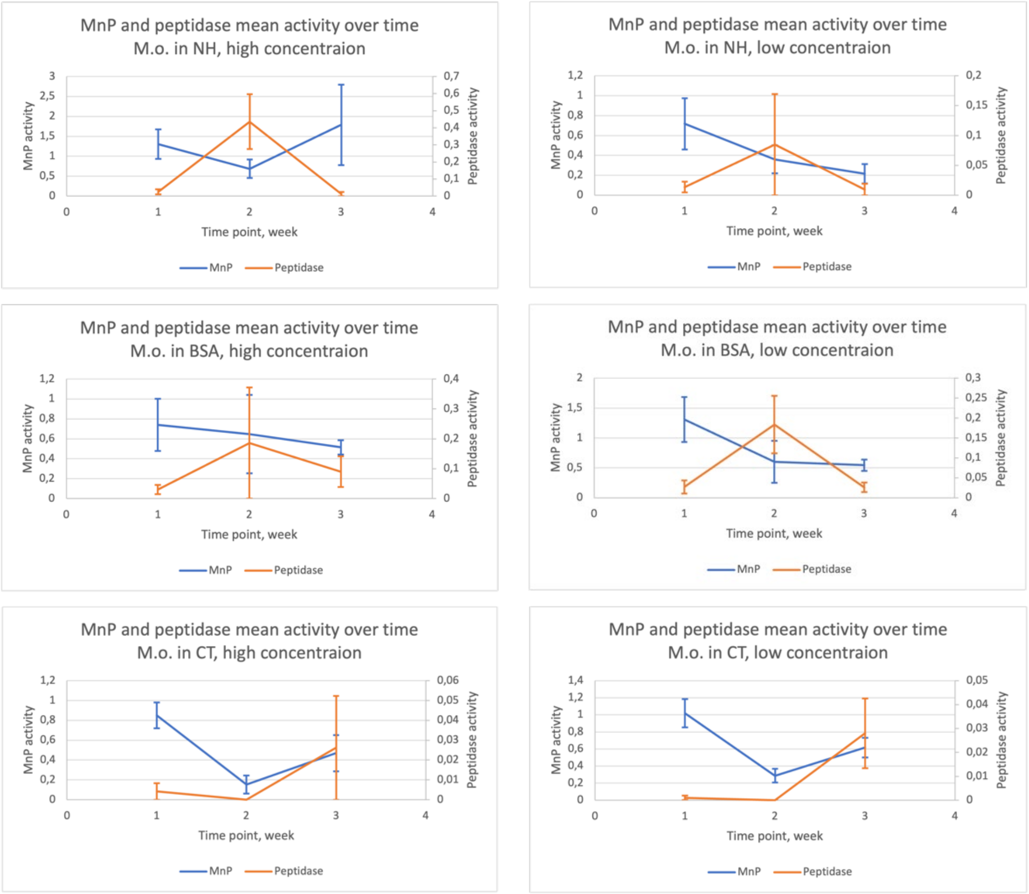


Figure 13. Peptidase activity of Marasmius in six mediums. Data points are grouped per treatment (NH_4, BSA, CT), concentration (high (12 mM), low (1.2. mM)) and sampling time (first, second, third). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Solid bars indicate pairs with significant difference in means from Tukey Test. Dotted bars indicate trends towards significance. Hollow circles of the same colour indicate trend towards significance. Text box lists significant differences not suitable for illustration.

Change in enzyme activities over time

Peptidase activity peaked at the second week for all mediums with readily or easily available N (NH_4, BSA) (Figure 14). Change in both MnP and peptidase activity over time was similar between mediums with low concentration of readily and easily available N (NH_4, BSA), where MnP activity decreased from the first time point. Great similarity could be seen between the two CT mediums, where both MnP and peptidase activity decreased from the first to the second time point, with a slight increase in MnP activity and a great increase in peptidase activity in the last sampling. In medium with No N, MnP activity peaked the second week of sampling, while peptidase activity dipped.



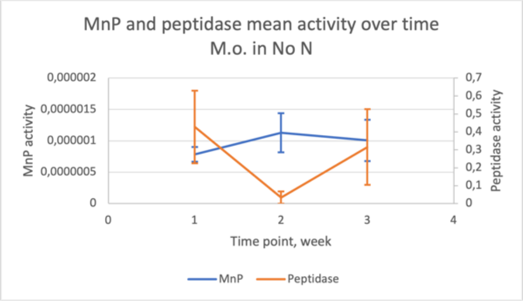


Figure 14. Mean MnP and peptidase activity of Marasmius in different mediums over time (weeks). MnP activity on left y-axis, "MnP", blue line. Peptidase activity on right y-axis, orange line. Scales of y-axes differ within and between mediums.

3.2 Biomass

3.2.1 *Hypholoma fasciculare*

Initial analysis of N control showed trend towards significance of N availability (p = 0.075) (Table 14) and Tukey Test showed slightly higher biomass of Hypholoma when grown in high nitrogen concentrations compared to when in low nitrogen concentrations (p = 0.068). There was no significant difference between No N and the others.

Table 14. Analysis of Variance of Hypholoma biomass in NH_4 and No N medium across concentration.

term	df	sumsq	meansq	statistic	p.value
N availability	2	1,134	0,567	3,178	**0,075**
Residuals	13	2,320	0,178		

Linear model of NH_4, BSA and CT showed significant difference between NH_4 and BSA (p = 0.03), and between concentrations high and low (p = 0.018). Trend towards significance was found between BSA high and CT low (p = 0.075). Analysis of variance (ANOVA) showed significant effect of medium (p = 0.038) and of concentration (p = 0.001), and trend towards significance in interaction of medium and concentration (p = 0.062) (Table 15).

Table 15. Analysis of Variance of Hypholoma biomass across mediums and concentration.

term	df	sumsq	meansq	statistic	p.value
Concentration	1	1,196	1,196	13,141	**0,001**
Medium	2	0,685	0,342	3,762	**0,038**
Concentration:Medium	2	0,570	0,285	3,132	**0,062**
Residuals	24	2,184	0,091		

Tukey test showed that mean biomass of replicates grown in high N concentration was significantly higher than those grown in low N concentration (p = 0.0014).

Specifically, biomass yield from high concentrations of NH_4 (highest biomass, 1.44 mg) was much greater than from low concentrations of both NH_4 ($p = 0.02$) and BSA ($p < 0.001$) (lowest biomass, 0.48). Generally, biomass from NH_4 medium was significantly higher than that from BSA ($p = 0.03$). Slightly higher biomass was measured in replicates given high concentrations of NH than those given low concentrations of CT ($p = 0.09$). See Appendix 2 for raw data.

In all, concentration of N generated bigger difference in biomass when it was in a more easy-to-reach state (NH_4, BSA) compared to when it was recalcitrant (CT), where mean biomass was similar between concentrations.

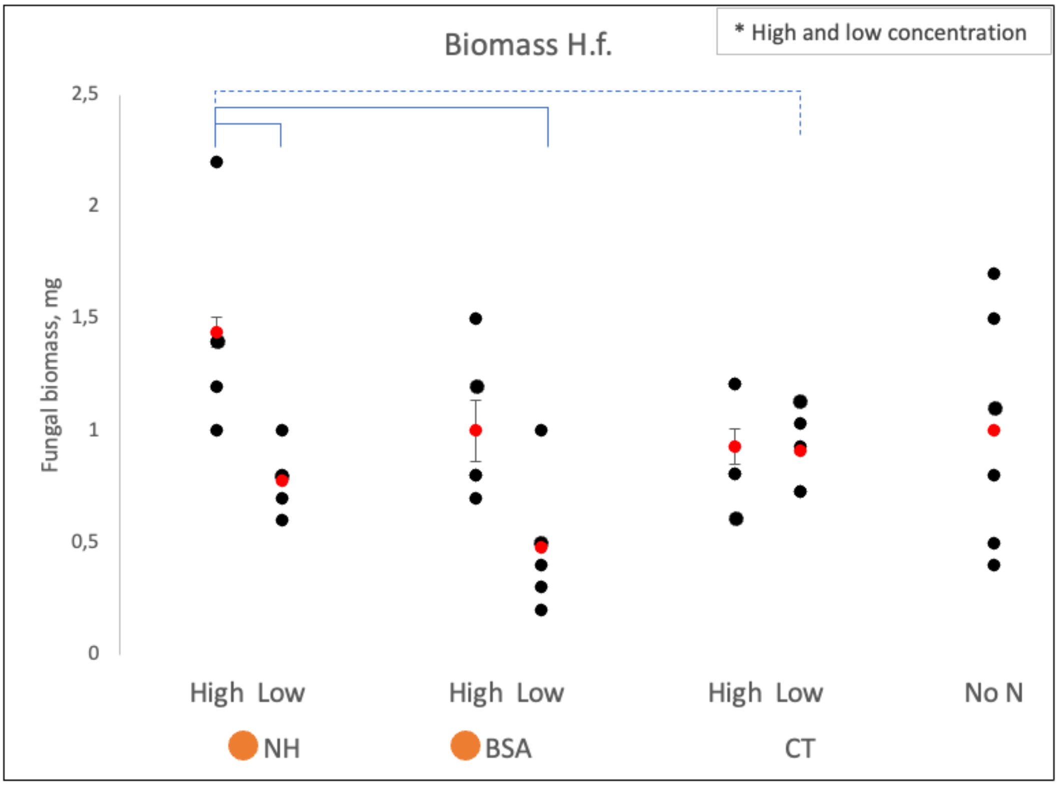


Figure 15. Biomass of Hypholoma in seven mediums. Data points are grouped per treatment (NH_4, BSA, CT) and concentration (high (12 mM), low (1.2. mM)). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Solid bars indicate pairs with significant difference in means from Tukey Test. Dotted bars indicate trends towards significance. Solid circles of the same colour indicate significant difference between mediums. Text box lists significant differences not suitable for illustration.

3.2.2 *Mycena epipterygia*

Initial analysis of N control showed no significant differences (Table 16).

Table 16. Analysis of Variance of Mycena biomass in NH_4 and No N medium across concentration.

term	df	sumsq	meansq	statistic	p.value
N availability	2	0,144	0,072	0,364	0,702
Residuals	13	2,580	0,198		

Linear model of NH_4, BSA and CT showed significant difference between concentrations high and low ($p < 0.001$), and between BSA high and NH_4 low ($p < 0.001$). Analysis of variance (ANOVA) showed significant effect of medium ($p = 0.03$), concentration ($p < 0.001$), and of interaction of medium and concentration ($p < 0.001$) (Table 17).

Table 17. Analysis of Variance of Mycena biomass across mediums and concentration.

term	df	sumsq	meansq	statistic	p.value
Concentration	2	0,948	0,474	4,075	**0,030**
Medium	1	5,976	5,976	51,370	**<0,001**
Concentration:Medium	2	2,613	1,306	11,230	**<0,001**
Residuals	24	2,792	0,116		

Tukey test showed that mean biomass of Mycena grown in CT medium was higher than that grown in BSA ($p = 0.029$). Biomass was significantly higher for replicates provided high concentration N than those provided low ($p < 0.001$), especially when nitrogen was in organic form (BSA) ($p < 0.001$) and when complexed with CT ($p < 0.001$). See Appendix 2 for raw data.

Biomass of Mycena was highest in the treatments with high concentration of CT (1.53 mg), and lowest in treatments with low concentration of BSA (-0.2 mg), with a significant difference between the two ($p = 0.028$).

In all, the effect of nitrogen concentration on biomass was greater when N was in a more difficult-to-reach state (BSA, CT) compared to when it was readily available (NH_4). In BSA and CT mediums, biomass was strongly impacted by what concentrations of the two that the fungi was given, with higher concentrations yielding larger biomass.

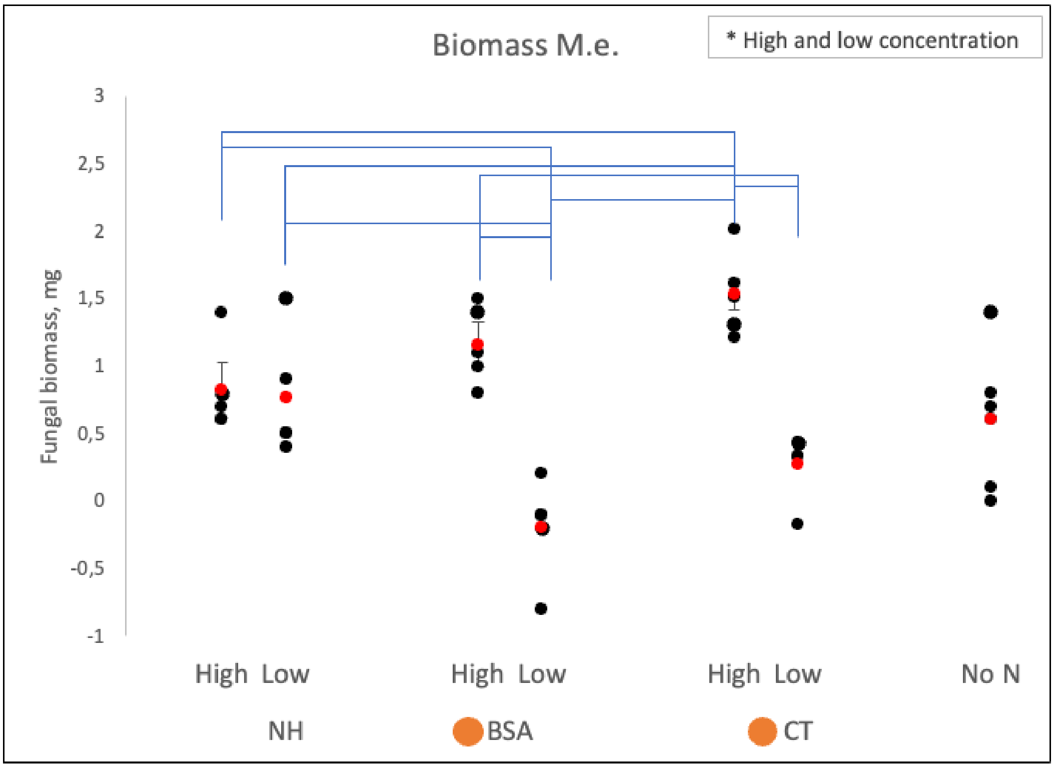


Figure 16. Biomass of Mycena in seven mediums. Data points are grouped per treatment (NH$_4$, BSA, CT) and concentration (high (12 mM), low (1.2. mM)). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Solid bars indicate pairs with significant difference in means from Tukey Test. Solid circles of the same colour indicate significant difference between mediums. Text box lists significant differences not suitable for illustration.

3.2.3 *Marasmius oreades*

Initial analysis of N control showed no significant differences (Table 18).

Table 18. Analysis of Variance of Marasmius biomass in NH$_4$ and No N medium across concentration.

term	df	sumsq	meansq	statistic	p.value
N availability	2	2,321	1,161	1,696	0,225
Residuals	12	8,212	0,684		

Linear model of NH$_4$, BSA and CT showed significant difference between CT and BSA ($p = 0.01$), and between BSA high and CT low ($p = 0.005$). Trend towards significance was found between BSA and CT ($p = 0.07$). Analysis of variance (ANOVA) showed significant effect of medium ($p < 0.001$) and of interaction of medium and concentration ($p = 0.018$) (Table 19).

Table 19. Analysis of Variance of Marasmius biomass across mediums and concentration.

term	df	sumsq	meansq	statistic	p.value
Concentration	2	4,050	2,025	13,743	**<0,001**

Medium	1	0,132	0,132	0,896	0,353
Concentration:Medium	2	1,403	0,701	4,761	**0,018**
Residuals	24	3,536	0,147		

Tukey test showed significant difference in biomass between NH_4 and BSA ($p = 0.001$) and between NH_4 and CT ($p < 0.001$), with NH_4 having the lower mass in both pairs. In CT medium, biomass was slightly higher in high concentration done in low concentrations ($p = 0.09$). Both high and low concentrations of NH_4 yielded lower biomass than both BSA low and CT high ($p = 0.022$, $p = 0.001$; $p = 0.008$, $p < 0.001$).
See Appendix 2 for raw data.

Mean biomass of Marasmius in all mediums was relatively high compared to Hypholoma and Mycena, ranging between 1.24 mg (NH_4 low) to 2.47 mg (CT high). Treatments with No N had a mean biomass higher than both NH_4 high, NH_4 low and BSA high.

In all, biomass was greater in more difficult-to-reach nutrient sources (BSA, CT, No N) compared to readily available N (NH_4). Higher N concentrations yielded higher biomass for NH_4 and CT, while the opposite was true for BSA. Greatest variation of biomass was found in Marasmius grown without nitrogen source (No N).

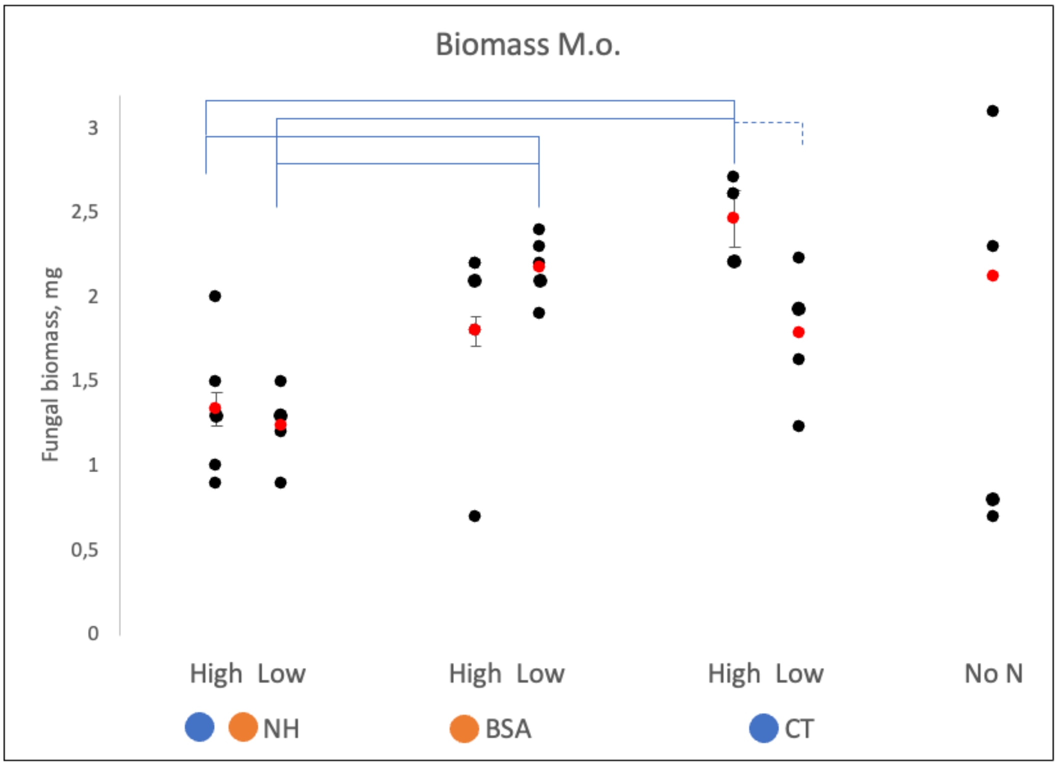


Figure 17. Biomass of Marasmius in seven mediums. Data points are grouped per treatment (NH_4, BSA, CT) and concentration (high (12 mM), low (1.2. mM)). Unique data points are shown in black, and the mean of each set is shown in red. Standard deviation is also included. Solid bars indicate pairs with significant difference in means from Tukey Test. Dotted bars indicate trends

towards significance. Solid circles of the same colour indicate significant difference between mediums. Text box lists significant differences not suitable for illustration.

4. Discussion

We hypothesized that fungi would adapt their enzyme activities to varying nitrogen sources, and that their biomass production would be impacted by what nitrogen source they were provided. Our hypothesis stated that mineral nitrogen would trigger insignificant levels of activity of both enzymes, that organic nitrogen would trigger high levels of peptidase activity, that tannin-complexed organic nitrogen would trigger high levels of manganese peroxidase (MnP) with a possible decrease over time in conjunction with increased peptidase as hydrolysable compounds were freed from complexations, and that complete nutrient starvations would trigger high Mn peroxidase activities. Biomass was expected to be lowest when under complete nitrogen starvation (No N medium) and highest when the fungi were provided high concentrations of readily available mineral nitrogen (NH_4). However, none of the fungi responded fully as we expected.

4.1 Fungal responses over time

Hypholoma fasciculare

Contrary to the hypothesis, MnP activity only increased between the first and last time point when the fungus was provided high concentration of readily available nitrogen (NH_4) and when provided no nitrogen at all (No N). MnP activity in replicates with high concentration of readily available N may not have reached its peak during the weeks of trial and would perhaps continue to increase. Meanwhile, MnP activity decreased in all other mediums between the first and last time point, including when nitrogen was complexed with condensed tannins. In response to high concentration of readily available NH_4, Hypholoma may have initially decreased enzyme activities in favor of biomass production (Figure 15). As nitrogen presumably became less available overtime the fungus may have been triggered to again increase its enzyme productions in response to starvation. However, changes in nutrient contents in the growth mediums was never assessed. In response to low concentrations of readily available nitrogen, Hypholoma initially maintained MnP expression, perhaps to explore if more nitrogen could be obtained, while decreasing peptidase activity. As peroxidase generated no additional nutrients and as its maintenance is energy-costly, Hypholoma seems to have shifted towards larger peptidase expression. In response to high concentrations of easily acquirable organic nitrogen (BSA), initial peptidase activity seems to have been enough to support biomass production (Figure 15), and as BSA possibly waned, peptidase activity increased again, perhaps to further meet nitrogen needs, while manganese peroxidase activity steadily decreased over time, possibly due to lack of triggering factors. In response to low concentrations of BSA, Hypholoma seems to have gone through a similar process as in high BSA concentrations, but at a slower pace, arguably due to the available nutrient-source being sparse, and did not reach a point of increase as it had yet more nitrogen to salvage. In mediums with recalcitrant nitrogen (CT), the shape of the graphs showing enzyme activities over time was as expected, where the presence of tannins stimulated MnP, which eventually decreased in conjunction with an increase in peptidase, possibly as the peptidase now had access to depolymerize the freed organic nitrogen. Yet, as discussed previously, MnP activities in CT:protein mediums was significantly lower than in the other mediums (NH_4, BSA). In response to complete nitrogen starvation (No N), Hypholoma increased both MnP and peptidase activity, supposedly in an attempt to find nitrogen.

Mycena epipterygia

For the fungus Mycena, trends of activities of manganese peroxidase and peptidase over time was similar between concentrations of each respective type of nitrogen (Figure 11). It is interesting to note that fungal response in mediums with organic nitrogen (BSA) was similar to that in mediums with mineral nitrogen (NH_4) regarding peptidase activity over time, while manganese peroxidase activity in BSA looked more alike that in mediums with tannin-complexed proteins (CT). BSA potentially triggered a reaction intermediate between the other two. Time was overall not a significant factor of Mycenas MnP activity in any medium, but instead, activity was governed by concentration of nitrogen, where higher nitrogen concentrations generated higher activity of MnP. Contrary, higher nitrogen concentrations generated lower peptidase activity, which overall varied significantly though time.

In response to high concentrations of readily available nitrogen (NH_4), Mycena slightly decreased its initially high MnP activity and maintained its peptidase activity low, possibly directing its energy towards building biomass. Just as for Hypholoma, Mycena may have quickly consumed much of the available nitrogen (not measured), which would explain the ensuing increase in both enzyme activities (Figure 11). In response to low concentrations of readily available nitrogen, MnP activity instead quickly ceased, whilst peptidase activity increased exponentially. The similarities in biomass production from the two NH_4 mediums (Figure 16) despite their large difference in nitrogen supply (12 mM and 1.2 mM respectively) may be attributed to how they allocated their energy to Mn peroxidase activity; if no resource was given MnP maintenance in low concentrations of NH_4 during the last two weeks, larger proportion of total nutrient intake could have been synthesized into fungal biomass, thus resulting in similar biomasses between concentrations. These findings are in correspondence with the theory that maintenance of Mn peroxidase activity through H_2O_2 production is very energy costly (Shimizu et al 2005). In contrast, biomass differed significantly between concentrations of both organic (BSA) and recalcitrant nitrogen (CT), while they displayed similar trends and levels of MnP activity (Figure 9, Figure 16). In mediums containing organic nitrogen, Mycena initially excreted both MnP and peptidase perhaps to explore the environment, shifting later to express mainly peptidase as this maybe gave the best return. In response to both high and low concentrations of recalcitrant nitrogen (CT), MnP peaked at similar levels of activity during the second week. As Mycena grew heaviest in mediums with high concentrations of recalcitrant nitrogen and as the low concentration counterpart showed an overall comparable trend, this suggests that the fungus successfully freed most complexed protein, suppressing the need for further peroxidase and efficiently utilizing the available nutrients. When provided no nitrogen at all, the steady decrease in peptidase activity parallel with the steady increase in manganese peroxidase supports the previously discussed starvation-induced peroxidase expression (Kirk & Tien 1988).

Marasmius oreades

As for Mycena, trends of Marasmius enzyme activities over time was similar between concentrations of each respective type of nitrogen (Figure 14). Just like for Mycena, trends of peptidase activity was similar between organic (BSA) and mineral (NH_4) nitrogen, while trends of manganese peroxidase activity was similar between BSA and recalcitrant nitrogen (CT), yet again implying BSA to invoke a reaction intermediate to NH_4 and BSA. As for Hypholoma, MnP activity increased from first to last time point only in high NH_4 concentrations and under complete nitrogen starvation (No N), contrary to hypothesis. Overall, there was no significant difference in MnP activity between mediums (Figure 12), while biomass from replicates grown in NH_4 mediums was significantly lower than those provided BSA and CT as a source of nitrogen (Figure 17).

MnP activity slightly decreased over time when Marasmius was growing in a high concentration of readily available NH_4 (Figure 13), possibly as there was no triggering factors such as starvation or presence of catabolic by-products. Sometime between the second and third time point, the environment may have become nitrogen depleted (not measured), leading again to an increase in MnP activity in an attempt to scavenge possible recalcitrant N. It is possible that MnP activity had not yet reached its peak in high concentration of NH_4 when the experiments ended. Low concentration of NH_4 resulted in decreased yet upheld MnP activity over time, and Marasmius spent some of its resources on peptidase expression. As the concentration of NH_4 were low, so was thus Marasmius' rate of enzyme production (Figure 14) as nitrogen is a vital component of proteins, yet it still sustained production of both. Also in replicates given organic nitrogen, peptidase activity increased after a week, but slightly less so than in mediums with inorganic nitrogen. Perhaps the production of peptidase was comparable between the two (NH_4, BSA) but as there was nothing to react with in NH_4, the enzymes were left unutilized and accumulated before they degraded. Just like in NH_4 mediums, peptidase activity in BSA mediums decreased from second to last time point, arguably due to the absence of peptidase-triggers. When nitrogen was complexed with tannins (CT), it seems like the MnP eventually released enough proteins to allow for subsequent hydrolysis.

4.2 Key findings

In most cases, enzyme activities were not affected by whether the provided nitrogen was mineral, part of a protein or if it was complexed with condensed tannins. Mycena showed no adaptation of either Mn peroxidase or peptidase activity to differing nutrient sources. A slight adaptation could be detected in Marasmius, as it responded with somewhat lower peptidase activity to condensed tannins than to both mineral and organic nitrogen ($p < 0.1$), yet MnP activity was unaffected.

Among the three species studied, Hypholoma stood out as the only one where the type of nitrogen had a notable impact ($p < 0.05$) on enzyme activities, namely manganese peroxidase. Curiously, contrary to our expectations, replicates supplied with proteins in tannin-complexation exhibited the lowest Mn peroxidase activity within this species. Nitrogen-deprived conditions have commonly been observed to induce high fungal MnP production, but in some exceptions, peroxidase activities have instead been stimulated by ample access to nitrogen and carbon (Rüttimann-Johnson et al 1994). The wood-associated and litter dwelling Hypholoma has previously been noted to efficiently utilize mineral nitrogen (Voříšková et al 2011), which may explain why it was in these mediums (NH_4) it grew the largest biomass.

Regarding the effects on biomass, the two other species showed different adaptation than Hypholoma. Both Mycena and Marasmius exhibited maximum growth when organic nitrogen was in complexation with tannin, suggesting an ability to exploit tannins as a source of carbon (Prigione et al 2018). Mycena is known for its capacity to efficiently decompose needles (Boberg et al 2011), which are rich in tannins (Hernes & Hedges 2004), and could potentially have retrieved enough nutrients with even low levels of MnP to utilize in biomass. Mycena and Marasmius had comparable biomass yield from mediums with organic nitrogen (BSA) and from mediums where the organic nitrogen was in complexation with condensed tannins (CT), indicating perhaps that tannin-complexation was not a significant restraint in their nutrient retrieval. Furthermore, replicates of these two species grown in mediums with mineral nitrogen (NH_4) grew similar in weight between concentrations, while biomass yield from mediums with organic and recalcitrant nitrogen showed evident effect of the concentration of which nitrogen had been provided. Again, this could possibly be in correlation to these fungi's manganese peroxidase responses, as MnP activity differed between concentrations of the two NH_4 mediums but not between the two BSA and CT mediums. It seems however that all three species were capable of growing in the presence of tannins.

4.3 Further research

The present study would be benefitted by supplementary measurements and analyses. For instance, continuously assessing nitrogen and carbon content in the growth medium might facilitate interpretation of enzymatic responses over time. Hypholoma and Marasmius grown in high concentrations of mineral nitrogen surprisingly had a strong increase in manganese peroxidase between the last two samplings. Knowledge about changes in nutrient concentrations would support the interpretation of fungal responses, and could clarify whether or not the late increase in manganese peroxidase expression was triggered by starvation.

With the data derived from laboratory experiments, there are additional possibilities of analysis. Response variables Mn peroxidase, peptidase and biomass were only tested in isolation, never in combination. For instance, as there in some cases was great variation of enzyme activities among replicates under identical conditions, and subsequent biomass within treatments varied as well, running statistical analysis on how individual replicates' biomass yield correlated with levels of the two enzyme activities would have been meaningful in order to further asses their correlation. Moreover, results would perhaps be more meaningful if the data from all seven mediums could have been analyzed together. In this study, the data from replicates containing no nitrogen (No N) were accounted for simply by relating the other data points to the mean value of No N, and by running a separate analysis on a dataset containing No N and mineral nitrogen (NH_4). Any significant difference between No N and organic/recalcitrant nitrogen was lost.

Concerning biomass yield, comparing the results from the studied saprotrophic basidiomycetes with the response of other types of fungi would have given interesting results. Initially, two species of Ascomycetes (*Penicillium spinulosum* and *Fusarium graminearum*) were included in the experiment, but these were later excluded due to time limitations. Had they remained, we would have a clearer picture on what role recalcitrant organic matter has on the forest floor regarding community compositions, as many species are unable to grow on condensed tannins (Prigione et al 2018). Another good control would have been to check if and how much mineral nitrogen was present in the Bovine serum albumin (BSA) used in this experiment as organic nitrogen source, to assess if the replicates provided BSA had access to inorganic nitrogen as well.

Expanding the number of variations in nutrient provisions could give valuable insight on the implications of e.g. forest fertilization. Additional replicates given combinations of mineral nitrogen and recalcitrant organic nitrogen would give a more representative image of real-life situations. In nature, fungal environments are rarely homogenic, and especially in the case of forest fertilization, various nutrient sources are present simultaneously. The present study only investigated the effect of nitrogen sources on manganese peroxidase, peptidase and biomass response from isolated cultures of three saprotrophic basidiomycetes. Contradicting results from previous research regarding changes in decomposition rate and overall fungal biomass following nutrient input (Jörgensen et al 2021, Okal et al 2020, Voříšková et al 2011, Janssens et al 2010, Kirk & Tien 1988) calls for more in-depth assessment of species-level studies. In some cases, species using peroxidases maintained their competitive advantage if one of the nutrients (e.g. phosphorus) remained limited. Yet other studies found opposing results, where fungal biomass remained same or even decreased following fertilization. This may in part be due to what the initial abiotic conditions were, if there was severe nutrient limitation or not (Jörgensen et al 2021).

On that note, it is worth mentioning that these fungi were grown under sterile and controlled laboratory conditions. While it may help to understand how these three saprotrophic basidiomycetes respond to variations in nutrient availability alone, it fails to take into consideration the multiple levels of factors present in the soil and the temporal changes in microbial community composition. As species composition in the soil is often a result of the long-term litter nutrient conditions, it is challenging to assess how specific species are affected by forced substrate modifications (Voříšková et al 2011). With other species gaining perhaps greater benefit from increased mineral nitrogen resources, saprotrophic basidiomycetes may quickly become outcompeted, as their specialization on nutrient acquisition from recalcitrant organic matter no longer provide them their competitive advantage. Field experiments have previously shown that increased fungal biomass following fertilization of boreal forests was a consequence of changed community compositions (Jörgensen et al 2021).

Furthermore, if fertilization reduce plant-nutrient conservation needs, tannin and other protective structures may over time become slightly less prevalent. Marginally more space could perhaps be available for occupancy for microorganisms harmed by tannins and constrained by recalcitrant matter, a small imbalance which perhaps could generate synergistic effect with nutrient-driven community changes, undermining our most important recyclers of nitrogen: saprotrophic basidiomycetes.

4.4 Considerations for Result Interpretation

Throughout this study, there were several aspects which may have contributed to flaws in the collected data. Both regarding the cultivation of the three species, sampling of the growth medium, analysis of enzyme activities and collection of biomass.

During the main experiment, fungi received no additional medium over the four week period before biomass collection. Some species seems to have incorporated most of the liquid, and at the end of the experiment, the Petri dish contained a gellike film around the glass beads, with little liquid left. If the liquid volume decreased over time it means that the enzyme concentrations then was relatively higher per volume which may have impacted results and perhaps showed larger relative activity. This may have skewed analysis regarding how activities changed over time. To account for this, the fungi could have been provided additional medium containing no nitrogen (No N). However, it would have been challenging to estimate what volume of liquid was actually left in each dish, and this could perhaps instead have resulted in a different skewedness.

As seen in chapter 3.1, enzyme activity often varied greatly within replicates. One explanation could be that the enzyme production is heterogenous throughout the mycelium, and that extractions of only small volumes of growth medium thus contained very different quantities of MnP and peptidase, which may have affected results. All datasets had a considerable amount of negative values, or activities below detection limit. This may be due to the trials running for too short or long, which could have been avoided if calibration tests were run. The negative values could also be due to a high noise to signal ratio. Another explanation could be that the samples taken from the mediums during the experiment contained some fungal hyphae which may have disturbed the analyses of enzyme activities in the machines.

Data of enzyme activities may have been inaccurate in some cases, as samples were not analyzed immediately. For example, about half of the analysis from the last sampling of Hypholoma was done after the summer, and there was an apparent difference between these and the ones run before summer started. Even as they were stored in -20°C, enzymes could have degraded. The difference was clear in this case, but other samples could have been affected as well. Some sets were run on the day of sampling, but some were kept in storage for multiple weeks. In future experiments, this could be avoided with better time planning or with more members on the team, able to conduct multiple tasks simultaneously.

The unexpected result in biomass could be due to the overall biomass being very low, and the weight scale used not being sensitive enough to capture differences I mycelial mass. Filter papers weighted around 1.5 g while the fungal biomass was in the order of milligrams. The negative values in Mycena could be due to that the filter papers were not entirely void of moisture during the first weight measurement, even after 48 hours of drying. This divergence could possibly apply to all other measurements as well.

5. Closing remarks

Responses to varying nitrogen sources and concentrations differed between the three saprotrophic basidiomycetes. All three adapted their enzyme production to their environment, albeit in surprising ways in some cases. How they adapted their enzyme activities over time was very different, and their biomass production in different mediums as well. None of the species met the initial expectations of behavior fully. While they are all saprotrophic basidiomycetes, their natural habitats differ, as Hypholoma is comfortable in wood and litter, Mycena specializes in needle decomposition and Marasmius is found mainly in grasslands. Our results show that these three fungal species will not remain indifferent to alterations of nutrient balances in their vicinity, and that fertilization in favor of carbon sequestration may indeed alter both the rate of decomposition and the community compositions. Furthermore, nitrogen supply greatly affected biomass production of all three species, and in some instances seem to have been tied to the activity of MnP. Studying only these three fungi, bearing similarities in their ability to retrieve nutrients from dead matter yet responding so adversely to nutrient sources under controlled laboratory conditions, it raises great concern and uncertainty to how microbial species will alter in composition as a response to anthropogenic interference in nutrient cycles. Yet, little information was obtained to support any strong argument regarding long-term effects of fertilization on these fungi and how they may in turn affect their surroundings. Here, they were cultivated under sterile and controlled conditions. In their natural habitat, competition is omnipresent and the fungi are impacted not only by their own response to abiotic factors, but also by other species and how these other species respond to abiotic factors themselves.

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Popular science summary

Saprotrophic basidiomycetes are known as our main decomposers of recalcitrant organic matter, such as lignin. They have the ability to use, in addition to hydrolytic enzymes, also oxidative enzymes which are highly efficient in breaking down complex macromolecules. Hence, their role in our major nutrient cycles are vital. Just like all other species, they exist in constant presence of competition and have adapted nutrient retrieval based on ancient and relatively stable nutrient and community balances. Anthropogenic interference alters both of these, and little is known about what effects on ecosystems will evoke from this. In this thesis, we investigate how three species of saprotrophic basidiomycetes respond to varying nitrogen sources. Under sterile laboratory conditions, the fungi were provided with high and low concentrations of readily available mineral nitrogen, easily accessible organic nitrogen and more difficult to reach nitrogen which was complexed with hard-to-digest plant material. Measurements were made over three weeks to determine activities of both hydrolytic enzymes and oxidative enzymes and their final biomass noted. Results showed that all three species responded differently from each other to all sources of nutrients, both regarding their enzyme activities and their biomass production.

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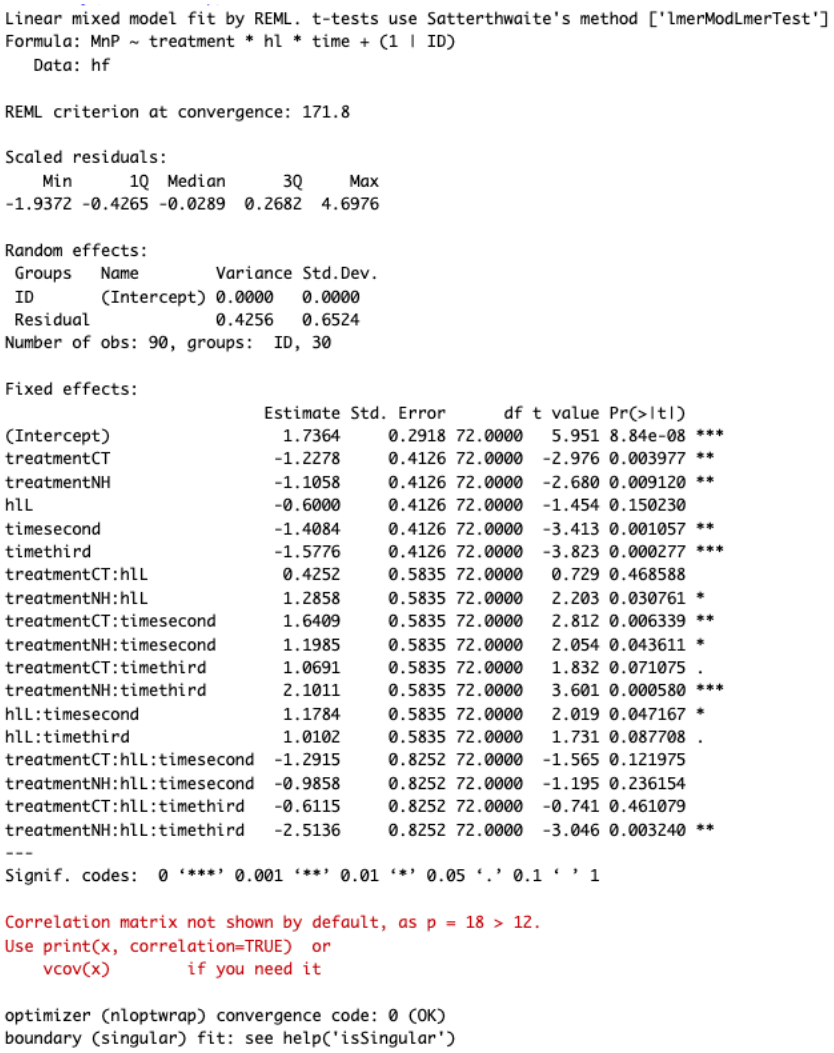
Appendix 1

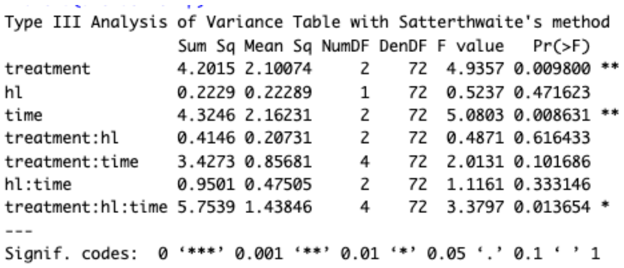
Pictures from analysis in RStudio of enzyme activities.

Hypholoma MnP



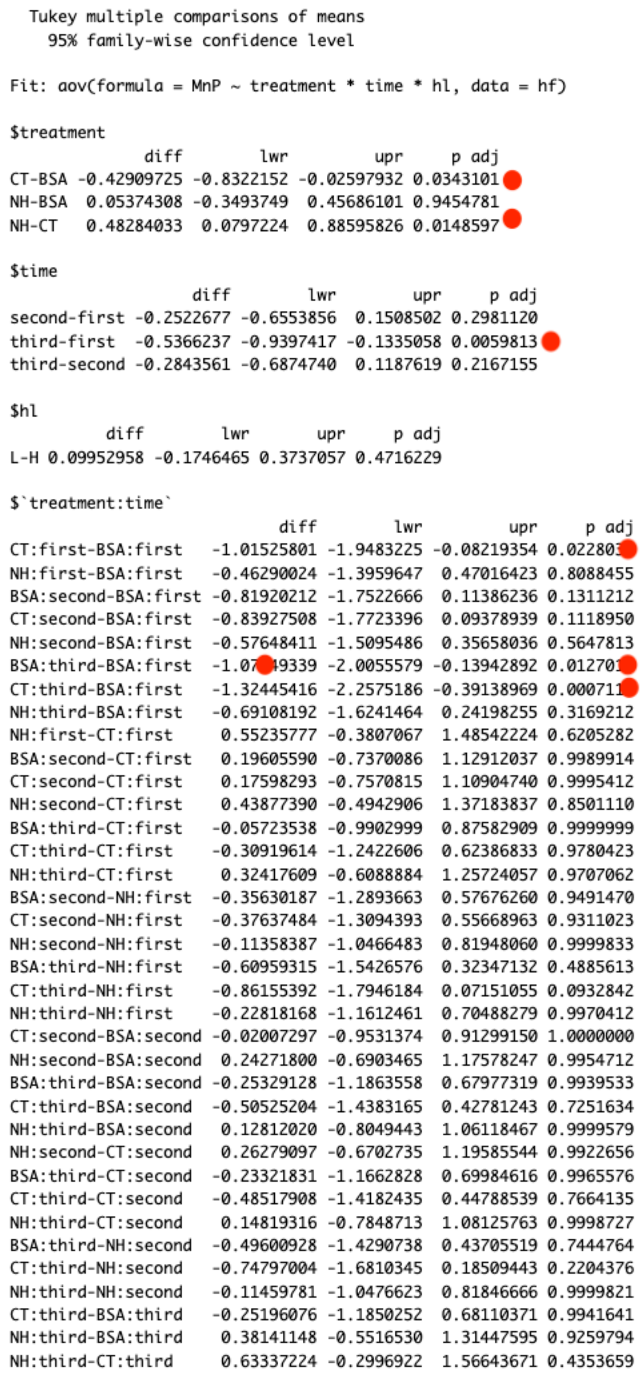
```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: MnP ~ treatment * hl * time + (1 | ID)
   Data: hf

REML criterion at convergence: 171.8

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-1.9372 -0.4265 -0.0289  0.2682  4.6976 

Random effects:
 Groups   Name        Variance Std.Dev.
 ID       (Intercept) 0.0000   0.0000  
 Residual             0.4256   0.6524  
Number of obs: 90, groups:  ID, 30

Fixed effects:
                           Estimate Std. Error      df t value Pr(>|t|)    
(Intercept)                  1.7364     0.2918 72.0000   5.951 8.84e-08 ***
treatmentCT                 -1.2278     0.4126 72.0000  -2.976 0.003977 ** 
treatmentNH                 -1.1058     0.4126 72.0000  -2.680 0.009120 ** 
hlL                         -0.6000     0.4126 72.0000  -1.454 0.150230    
timesecond                  -1.4084     0.4126 72.0000  -3.413 0.001057 ** 
timethird                   -1.5776     0.4126 72.0000  -3.823 0.000277 ***
treatmentCT:hlL              0.4252     0.5835 72.0000   0.729 0.468588    
treatmentNH:hlL              1.2858     0.5835 72.0000   2.203 0.030761 *  
treatmentCT:timesecond       1.6409     0.5835 72.0000   2.812 0.006339 ** 
treatmentNH:timesecond       1.1985     0.5835 72.0000   2.054 0.043611 *  
treatmentCT:timethird        1.0691     0.5835 72.0000   1.832 0.071075 .  
treatmentNH:timethird        2.1011     0.5835 72.0000   3.601 0.000580 ***
hlL:timesecond               1.1784     0.5835 72.0000   2.019 0.047167 *  
hlL:timethird                1.0102     0.5835 72.0000   1.731 0.087708 .  
treatmentCT:hlL:timesecond  -1.2915     0.8252 72.0000  -1.565 0.121975    
treatmentNH:hlL:timesecond  -0.9858     0.8252 72.0000  -1.195 0.236154    
treatmentCT:hlL:timethird   -0.6115     0.8252 72.0000  -0.741 0.461079    
treatmentNH:hlL:timethird   -2.5136     0.8252 72.0000  -3.046 0.003240 ** 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation matrix not shown by default, as p = 18 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it

optimizer (nloptwrap) convergence code: 0 (OK)
boundary (singular) fit: see help('isSingular')
```

Figure 18. Linear mixed model of MnP activity in Hypholoma, interactions time, medium and concentration.

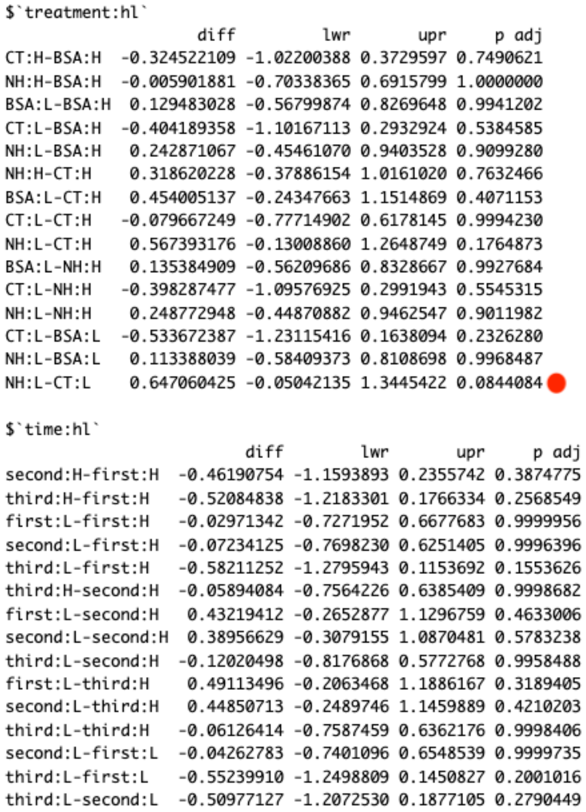


```
Type III Analysis of Variance Table with Satterthwaite's method
                  Sum Sq Mean Sq NumDF DenDF F value   Pr(>F)
treatment         4.2015 2.10074     2    72  4.9357 0.009800 **
hl                0.2229 0.22289     1    72  0.5237 0.471623
time              4.3246 2.16231     2    72  5.0803 0.008631 **
treatment:hl      0.4146 0.20731     2    72  0.4871 0.616433
treatment:time    3.4273 0.85681     4    72  2.0131 0.101686
hl:time           0.9501 0.47505     2    72  1.1161 0.333146
treatment:hl:time 5.7539 1.43846     4    72  3.3797 0.013654 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

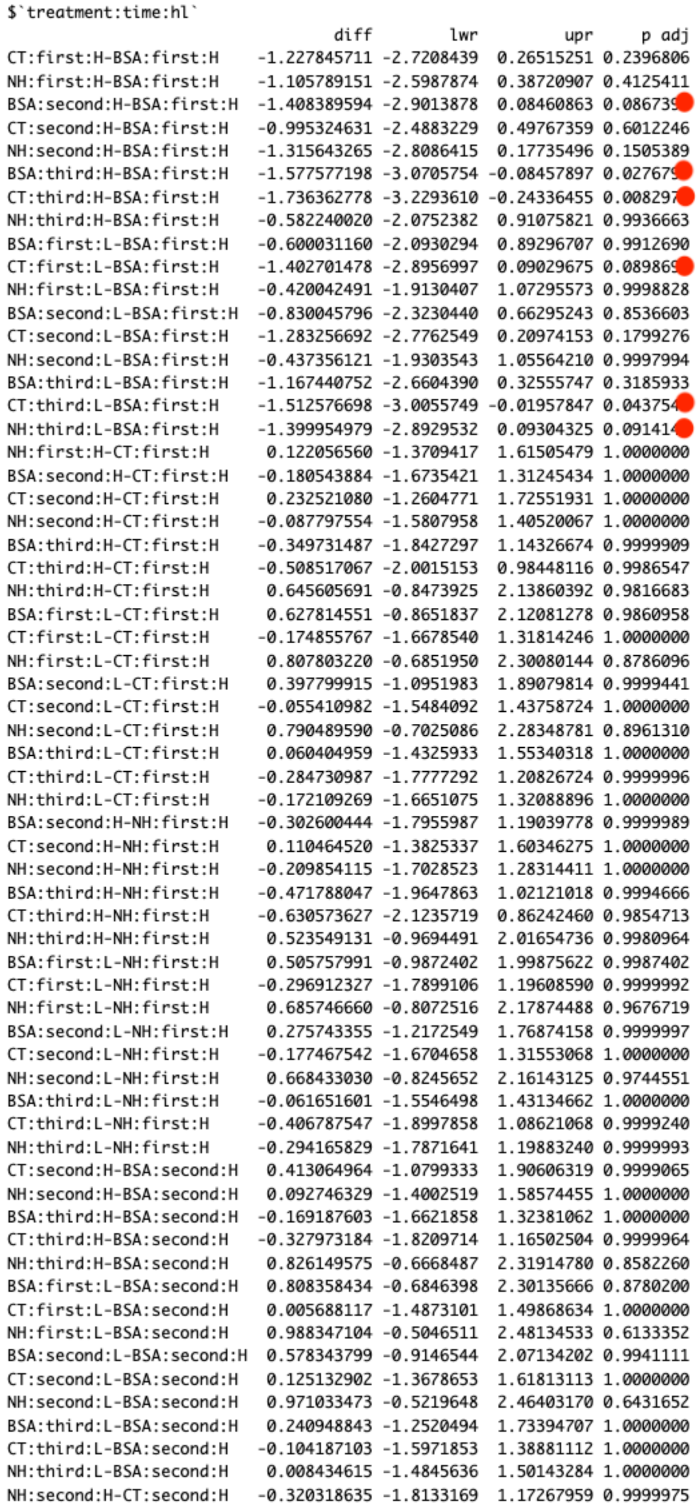
Figure 19. ANOVA of MnP activity in Hypholoma, interactions time, medium and concentration.



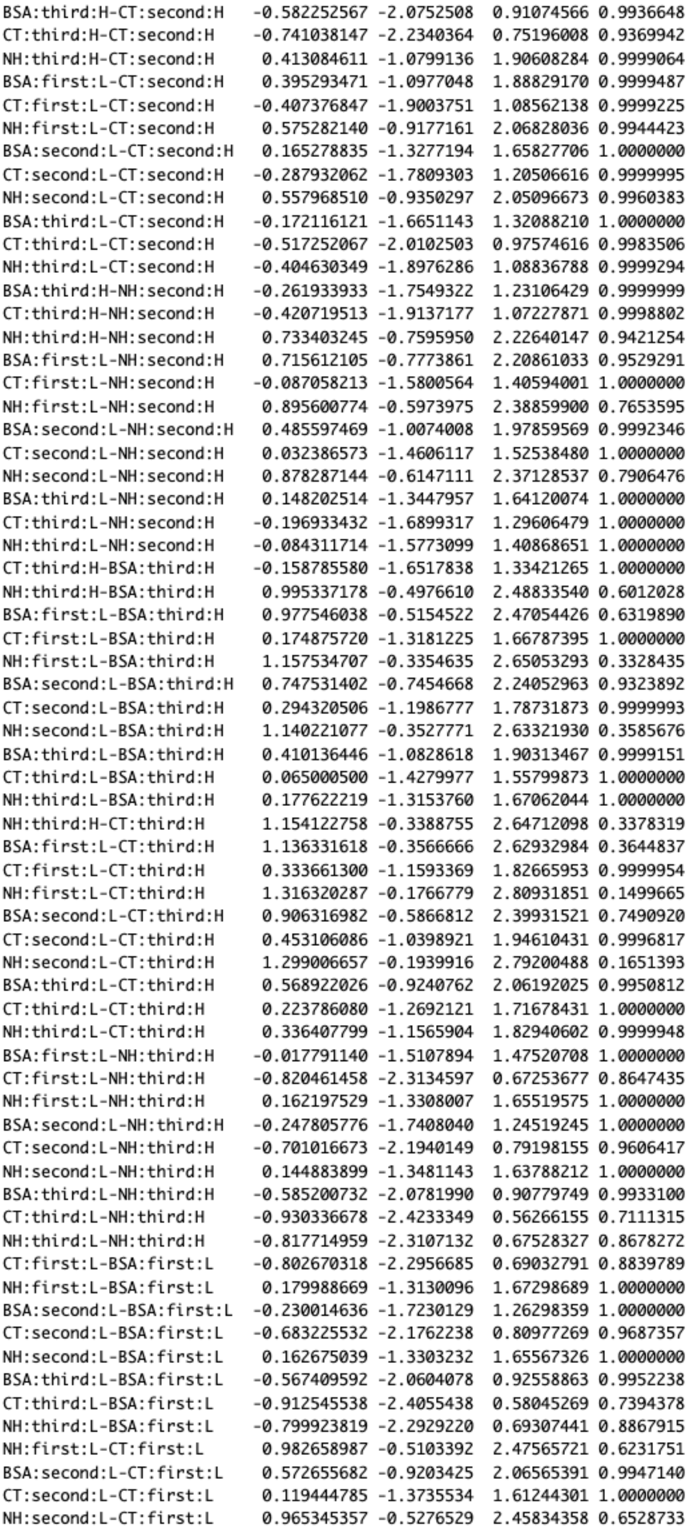
```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = MnP ~ treatment * time * hl, data = hf)

$treatment
              diff        lwr         upr     p adj
CT-BSA -0.42909725 -0.8322152 -0.02597932 0.0343101●
NH-BSA  0.05374308 -0.3493749  0.45686101 0.9454781
NH-CT   0.48284033  0.0797224  0.88595826 0.0148597●

$time
                   diff        lwr        upr     p adj
second-first -0.2522677 -0.6553856  0.1508502 0.2981120
third-first  -0.5366237 -0.9397417 -0.1335058 0.0059813●
third-second -0.2843561 -0.6874740  0.1187619 0.2167155

$hl
          diff        lwr       upr     p adj
L-H 0.09952958 -0.1746465 0.3737057 0.4716229

$`treatment:time`
                            diff        lwr         upr     p adj
CT:first-BSA:first   -1.01525801 -1.9483225 -0.08219354 0.022803●
NH:first-BSA:first   -0.46290024 -1.3959647  0.47016423 0.8088455
BSA:second-BSA:first -0.81920212 -1.7522666  0.11386236 0.1311212
CT:second-BSA:first  -0.83927508 -1.7723396  0.09378939 0.1118950
NH:second-BSA:first  -0.57648411 -1.5095486  0.35658036 0.5647813
BSA:third-BSA:first  -1.07●49339 -2.0055579 -0.13942892 0.012701●
CT:third-BSA:first   -1.32445416 -2.2575186 -0.39138969 0.000711●
NH:third-BSA:first   -0.69108192 -1.6241464  0.24198255 0.3169212
NH:first-CT:first     0.55235777 -0.3807067  1.48542224 0.6205282
BSA:second-CT:first   0.19605590 -0.7370086  1.12912037 0.9989914
CT:second-CT:first    0.17598293 -0.7570815  1.10904740 0.9995412
NH:second-CT:first    0.43877390 -0.4942906  1.37183837 0.8501110
BSA:third-CT:first   -0.05723538 -0.9902999  0.87582909 0.9999999
CT:third-CT:first    -0.30919614 -1.2422606  0.62386833 0.9780423
NH:third-CT:first     0.32417609 -0.6088884  1.25724057 0.9707062
BSA:second-NH:first  -0.35630187 -1.2893663  0.57676260 0.9491470
CT:second-NH:first   -0.37637484 -1.3094393  0.55668963 0.9311023
NH:second-NH:first   -0.11358387 -1.0466483  0.81948060 0.9999833
BSA:third-NH:first   -0.60959315 -1.5426576  0.32347132 0.4885613
CT:third-NH:first    -0.86155392 -1.7946184  0.07151055 0.0932842
NH:third-NH:first    -0.22818168 -1.1612461  0.70488279 0.9970412
CT:second-BSA:second -0.02007297 -0.9531374  0.91299150 1.0000000
NH:second-BSA:second  0.24271800 -0.6903465  1.17578247 0.9954712
BSA:third-BSA:second -0.25329128 -1.1863558  0.67977319 0.9939533
CT:third-BSA:second  -0.50525204 -1.4383165  0.42781243 0.7251634
NH:third-BSA:second   0.12812020 -0.8049443  1.06118467 0.9999579
NH:second-CT:second   0.26279097 -0.6702735  1.19585544 0.9922656
BSA:third-CT:second  -0.23321831 -1.1662828  0.69984616 0.9965576
CT:third-CT:second   -0.48517908 -1.4182435  0.44788539 0.7664135
NH:third-CT:second    0.14819316 -0.7848713  1.08125763 0.9998727
BSA:third-NH:second  -0.49600928 -1.4290738  0.43705519 0.7444764
CT:third-NH:second   -0.74797004 -1.6810345  0.18509443 0.2204376
NH:third-NH:second   -0.11459781 -1.0476623  0.81846666 0.9999821
CT:third-BSA:third   -0.25196076 -1.1850252  0.68110371 0.9941641
NH:third-BSA:third    0.38141148 -0.5516530  1.31447595 0.9259794
NH:third-CT:third     0.63337224 -0.2996922  1.56643671 0.4353659
```



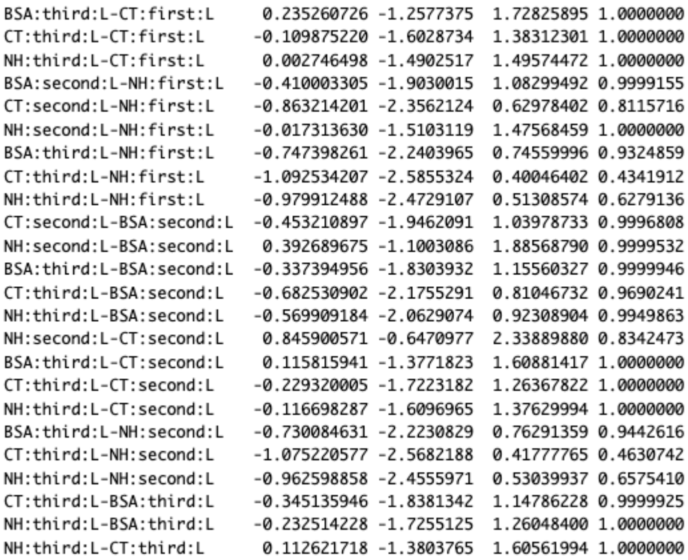
```
$`treatment:hl`
                    diff         lwr       upr     p adj
CT:H-BSA:H  -0.324522109 -1.02200388 0.3729597 0.7490621
NH:H-BSA:H  -0.005901881 -0.70338365 0.6915799 1.0000000
BSA:L-BSA:H  0.129483028 -0.56799874 0.8269648 0.9941202
CT:L-BSA:H  -0.404189358 -1.10167113 0.2932924 0.5384585
NH:L-BSA:H   0.242871067 -0.45461070 0.9403528 0.9099280
NH:H-CT:H    0.318620228 -0.37886154 1.0161020 0.7632466
BSA:L-CT:H   0.454005137 -0.24347663 1.1514869 0.4071153
CT:L-CT:H   -0.079667249 -0.77714902 0.6178145 0.9994230
NH:L-CT:H    0.567393176 -0.13008860 1.2648749 0.1764873
BSA:L-NH:H   0.135384909 -0.56209686 0.8328667 0.9927684
CT:L-NH:H   -0.398287477 -1.09576925 0.2991943 0.5545315
NH:L-NH:H    0.248772948 -0.44870882 0.9462547 0.9011982
CT:L-BSA:L  -0.533672387 -1.23115416 0.1638094 0.2326280
NH:L-BSA:L   0.113388039 -0.58409373 0.8108698 0.9968487
NH:L-CT:L    0.647060425 -0.05042135 1.3445422 0.0844084 ●

$`time:hl`
                         diff        lwr       upr     p adj
second:H-first:H  -0.46190754 -1.1593893 0.2355742 0.3874775
third:H-first:H   -0.52084838 -1.2183301 0.1766334 0.2568549
first:L-first:H   -0.02971342 -0.7271952 0.6677683 0.9999956
second:L-first:H  -0.07234125 -0.7698230 0.6251405 0.9996396
third:L-first:H   -0.58211252 -1.2795943 0.1153692 0.1553626
third:H-second:H  -0.05894084 -0.7564226 0.6385409 0.9998682
first:L-second:H   0.43219412 -0.2652877 1.1296759 0.4633006
second:L-second:H  0.38956629 -0.3079155 1.0870481 0.5783238
third:L-second:H  -0.12020498 -0.8176868 0.5772768 0.9958488
first:L-third:H    0.49113496 -0.2063468 1.1886167 0.3189405
second:L-third:H   0.44850713 -0.2489746 1.1459889 0.4210203
third:L-third:H   -0.06126414 -0.7587459 0.6362176 0.9998406
second:L-first:L  -0.04262783 -0.7401096 0.6548539 0.9999735
third:L-first:L   -0.55239910 -1.2498809 0.1450827 0.2001016
third:L-second:L  -0.50977127 -1.2072530 0.1877105 0.2790449
```



```
$`treatment:time:hl`
                                diff        lwr         upr     p adj
CT:first:H-BSA:first:H     -1.227845711 -2.7208439  0.26515251 0.2396806
NH:first:H-BSA:first:H     -1.105789151 -2.5987874  0.38720907 0.4125411
BSA:second:H-BSA:first:H   -1.408389594 -2.9013878  0.08460863 0.086739●
CT:second:H-BSA:first:H    -0.995324631 -2.4883229  0.49767359 0.6012246
NH:second:H-BSA:first:H    -1.315643265 -2.8086415  0.17735496 0.1505389
BSA:third:H-BSA:first:H    -1.577577198 -3.0705754 -0.08457897 0.027679●
CT:third:H-BSA:first:H     -1.736362778 -3.2293610 -0.24336455 0.008297●
NH:third:H-BSA:first:H     -0.582240020 -2.0752382  0.91075821 0.9936663
BSA:first:L-BSA:first:H    -0.600031160 -2.0930294  0.89296707 0.9912690
CT:first:L-BSA:first:H     -1.402701478 -2.8956997  0.09029675 0.089869●
NH:first:L-BSA:first:H     -0.420042491 -1.9130407  1.07295573 0.9998828
BSA:second:L-BSA:first:H   -0.830045796 -2.3230440  0.66295243 0.8536603
CT:second:L-BSA:first:H    -1.283256692 -2.7762549  0.20974153 0.1799276
NH:second:L-BSA:first:H    -0.437356121 -1.9303543  1.05564210 0.9997994
BSA:third:L-BSA:first:H    -1.167440752 -2.6604390  0.32555747 0.3185933
CT:third:L-BSA:first:H     -1.512576698 -3.0055749 -0.01957847 0.043754●
NH:third:L-BSA:first:H     -1.399954979 -2.8929532  0.09304325 0.091414●
NH:first:H-CT:first:H       0.122056560 -1.3709417  1.61505479 1.0000000
BSA:second:H-CT:first:H    -0.180543884 -1.6735421  1.31245434 1.0000000
CT:second:H-CT:first:H      0.232521080 -1.2604771  1.72551931 1.0000000
NH:second:H-CT:first:H     -0.087797554 -1.5807958  1.40520067 1.0000000
BSA:third:H-CT:first:H     -0.349731487 -1.8427297  1.14326674 0.9999909
CT:third:H-CT:first:H      -0.508517067 -2.0015153  0.98448116 0.9986547
NH:third:H-CT:first:H       0.645605691 -0.8473925  2.13860392 0.9816683
BSA:first:L-CT:first:H      0.627814551 -0.8651837  2.12081278 0.9860958
CT:first:L-CT:first:H      -0.174855767 -1.6678540  1.31814246 1.0000000
NH:first:L-CT:first:H       0.807803220 -0.6851950  2.30080144 0.8786096
BSA:second:L-CT:first:H     0.397799915 -1.0951983  1.89079814 0.9999441
CT:second:L-CT:first:H     -0.055410982 -1.5484092  1.43758724 1.0000000
NH:second:L-CT:first:H      0.790489590 -0.7025086  2.28348781 0.8961310
BSA:third:L-CT:first:H      0.060404959 -1.4325933  1.55340318 1.0000000
CT:third:L-CT:first:H      -0.284730987 -1.7777292  1.20826724 0.9999996
NH:third:L-CT:first:H      -0.172109269 -1.6651075  1.32088896 1.0000000
BSA:second:H-NH:first:H    -0.302600444 -1.7955987  1.19039778 0.9999989
CT:second:H-NH:first:H      0.110464520 -1.3825337  1.60346275 1.0000000
NH:second:H-NH:first:H     -0.209854115 -1.7028523  1.28314411 1.0000000
BSA:third:H-NH:first:H     -0.471788047 -1.9647863  1.02121018 0.9994666
CT:third:H-NH:first:H      -0.630573627 -2.1235719  0.86242460 0.9854713
NH:third:H-NH:first:H       0.523549131 -0.9694491  2.01654736 0.9980964
BSA:first:L-NH:first:H      0.505757991 -0.9872402  1.99875622 0.9987402
CT:first:L-NH:first:H      -0.296912327 -1.7899106  1.19608590 0.9999992
NH:first:L-NH:first:H       0.685746660 -0.8072516  2.17874488 0.9676719
BSA:second:L-NH:first:H     0.275743355 -1.2172549  1.76874158 0.9999997
CT:second:L-NH:first:H     -0.177467542 -1.6704658  1.31553068 1.0000000
NH:second:L-NH:first:H      0.668433030 -0.8245652  2.16143125 0.9744551
BSA:third:L-NH:first:H     -0.061651601 -1.5546498  1.43134662 1.0000000
CT:third:L-NH:first:H      -0.406787547 -1.8997858  1.08621068 0.9999240
NH:third:L-NH:first:H      -0.294165829 -1.7871641  1.19883240 0.9999993
CT:second:H-BSA:second:H    0.413064964 -1.0799333  1.90606319 0.9999065
NH:second:H-BSA:second:H    0.092746329 -1.4002519  1.58574455 1.0000000
BSA:third:H-BSA:second:H   -0.169187603 -1.6621858  1.32381062 1.0000000
CT:third:H-BSA:second:H    -0.327973184 -1.8209714  1.16502504 0.9999964
NH:third:H-BSA:second:H     0.826149575 -0.6668487  2.31914780 0.8582260
BSA:first:L-BSA:second:H    0.808358434 -0.6846398  2.30135666 0.8780200
CT:first:L-BSA:second:H     0.005688117 -1.4873101  1.49868634 1.0000000
NH:first:L-BSA:second:H     0.988347104 -0.5046511  2.48134533 0.6133352
BSA:second:L-BSA:second:H   0.578343799 -0.9146544  2.07134202 0.9941111
CT:second:L-BSA:second:H    0.125132902 -1.3678653  1.61813113 1.0000000
NH:second:L-BSA:second:H    0.971033473 -0.5219648  2.46403170 0.6431652
BSA:third:L-BSA:second:H    0.240948843 -1.2520494  1.73394707 1.0000000
CT:third:L-BSA:second:H    -0.104187103 -1.5971853  1.38881112 1.0000000
NH:third:L-BSA:second:H     0.008434615 -1.4845636  1.50143284 1.0000000
NH:second:H-CT:second:H    -0.320318635 -1.8133169  1.17267959 0.9999975
```



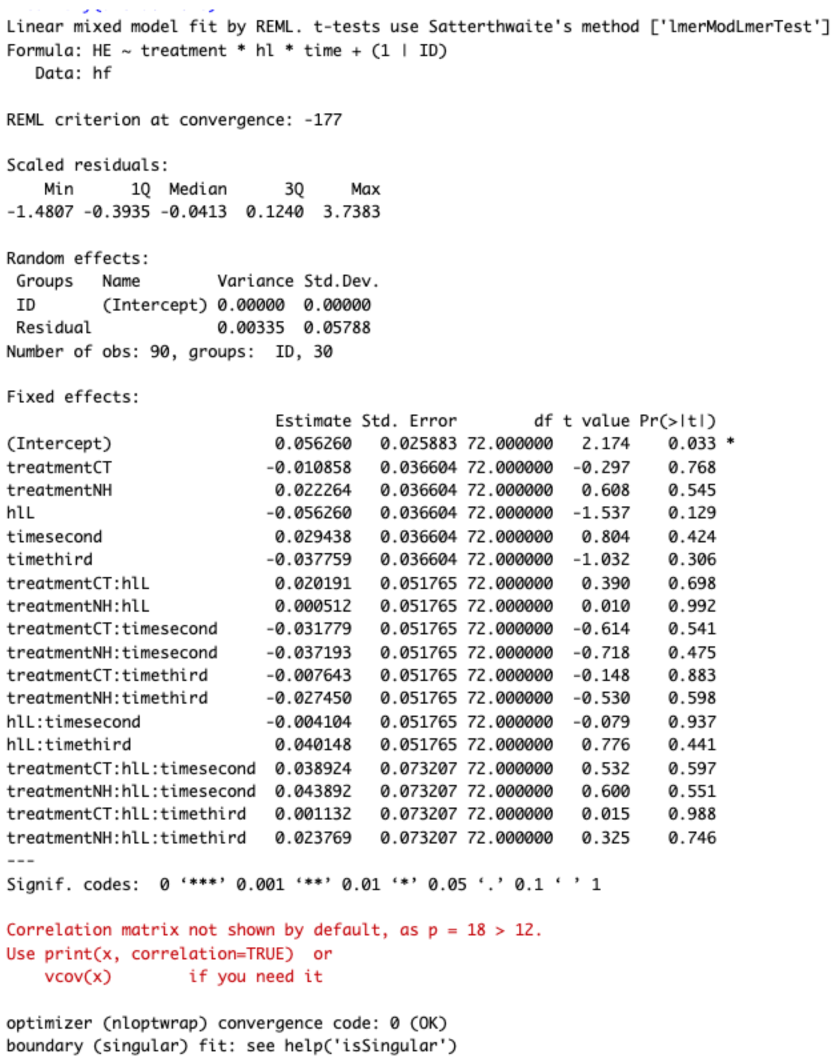
```
BSA:third:H-CT:second:H   -0.582252567 -2.0752508  0.91074566 0.9936648
CT:third:H-CT:second:H    -0.741038147 -2.2340364  0.75196008 0.9369942
NH:third:H-CT:second:H     0.413084611 -1.0799136  1.90608284 0.9999064
BSA:first:L-CT:second:H    0.395293471 -1.0977048  1.88829170 0.9999487
CT:first:L-CT:second:H    -0.407376847 -1.9003751  1.08562138 0.9999225
NH:first:L-CT:second:H     0.575282140 -0.9177161  2.06828036 0.9944423
BSA:second:L-CT:second:H   0.165278835 -1.3277194  1.65827706 1.0000000
CT:second:L-CT:second:H   -0.287932062 -1.7809303  1.20506616 0.9999995
NH:second:L-CT:second:H    0.557968510 -0.9350297  2.05096673 0.9960383
BSA:third:L-CT:second:H   -0.172116121 -1.6651143  1.32088210 1.0000000
CT:third:L-CT:second:H    -0.517252067 -2.0102503  0.97574616 0.9983506
NH:third:L-CT:second:H    -0.404630349 -1.8976286  1.08836788 0.9999294
BSA:third:H-NH:second:H   -0.261933933 -1.7549322  1.23106429 0.9999999
CT:third:H-NH:second:H    -0.420719513 -1.9137177  1.07227871 0.9998802
NH:third:H-NH:second:H     0.733403245 -0.7595950  2.22640147 0.9421254
BSA:first:L-NH:second:H    0.715612105 -0.7773861  2.20861033 0.9529291
CT:first:L-NH:second:H    -0.087058213 -1.5800564  1.40594001 1.0000000
NH:first:L-NH:second:H     0.895600774 -0.5973975  2.38859900 0.7653595
BSA:second:L-NH:second:H   0.485597469 -1.0074008  1.97859569 0.9992346
CT:second:L-NH:second:H    0.032386573 -1.4606117  1.52538480 1.0000000
NH:second:L-NH:second:H    0.878287144 -0.6147111  2.37128537 0.7906476
BSA:third:L-NH:second:H    0.148202514 -1.3447957  1.64120074 1.0000000
CT:third:L-NH:second:H    -0.196933432 -1.6899317  1.29606479 1.0000000
NH:third:L-NH:second:H    -0.084311714 -1.5773099  1.40868651 1.0000000
CT:third:H-BSA:third:H    -0.158785580 -1.6517838  1.33421265 1.0000000
NH:third:H-BSA:third:H     0.995337178 -0.4976610  2.48833540 0.6012028
BSA:first:L-BSA:third:H    0.977546038 -0.5154522  2.47054426 0.6319890
CT:first:L-BSA:third:H     0.174875720 -1.3181225  1.66787395 1.0000000
NH:first:L-BSA:third:H     1.157534707 -0.3354635  2.65053293 0.3328435
BSA:second:L-BSA:third:H   0.747531402 -0.7454668  2.24052963 0.9323892
CT:second:L-BSA:third:H    0.294320506 -1.1986777  1.78731873 0.9999993
NH:second:L-BSA:third:H    1.140221077 -0.3527771  2.63321930 0.3585676
BSA:third:L-BSA:third:H    0.410136446 -1.0828618  1.90313467 0.9999151
CT:third:L-BSA:third:H     0.065000500 -1.4279977  1.55799873 1.0000000
NH:third:L-BSA:third:H     0.177622219 -1.3153760  1.67062044 1.0000000
NH:third:H-CT:third:H      1.154122758 -0.3388755  2.64712098 0.3378319
BSA:first:L-CT:third:H     1.136331618 -0.3566666  2.62932984 0.3644837
CT:first:L-CT:third:H      0.333661300 -1.1593369  1.82665953 0.9999954
NH:first:L-CT:third:H      1.316320287 -0.1766779  2.80931851 0.1499665
BSA:second:L-CT:third:H    0.906316982 -0.5866812  2.39931521 0.7490920
CT:second:L-CT:third:H     0.453106086 -1.0398921  1.94610431 0.9996817
NH:second:L-CT:third:H     1.299006657 -0.1939916  2.79200488 0.1651393
BSA:third:L-CT:third:H     0.568922026 -0.9240762  2.06192025 0.9950812
CT:third:L-CT:third:H      0.223786080 -1.2692121  1.71678431 1.0000000
NH:third:L-CT:third:H      0.336407799 -1.1565904  1.82940602 0.9999948
BSA:first:L-NH:third:H    -0.017791140 -1.5107894  1.47520708 1.0000000
CT:first:L-NH:third:H     -0.820461458 -2.3134597  0.67253677 0.8647435
NH:first:L-NH:third:H      0.162197529 -1.3308007  1.65519575 1.0000000
BSA:second:L-NH:third:H   -0.247805776 -1.7408040  1.24519245 1.0000000
CT:second:L-NH:third:H    -0.701016673 -2.1940149  0.79198155 0.9606417
NH:second:L-NH:third:H     0.144883899 -1.3481143  1.63788212 1.0000000
BSA:third:L-NH:third:H    -0.585200732 -2.0781990  0.90779749 0.9933100
CT:third:L-NH:third:H     -0.930336678 -2.4233349  0.56266155 0.7111315
NH:third:L-NH:third:H     -0.817714959 -2.3107132  0.67528327 0.8678272
CT:first:L-BSA:first:L    -0.802670318 -2.2956685  0.69032791 0.8839789
NH:first:L-BSA:first:L     0.179988669 -1.3130096  1.67298689 1.0000000
BSA:second:L-BSA:first:L  -0.230014636 -1.7230129  1.26298359 1.0000000
CT:second:L-BSA:first:L   -0.683225532 -2.1762238  0.80977269 0.9687357
NH:second:L-BSA:first:L    0.162675039 -1.3303232  1.65567326 1.0000000
BSA:third:L-BSA:first:L   -0.567409592 -2.0604078  0.92558863 0.9952238
CT:third:L-BSA:first:L    -0.912545538 -2.4055438  0.58045269 0.7394378
NH:third:L-BSA:first:L    -0.799923819 -2.2929220  0.69307441 0.8867915
NH:first:L-CT:first:L      0.982658987 -0.5103392  2.47565721 0.6231751
BSA:second:L-CT:first:L    0.572655682 -0.9203425  2.06565391 0.9947140
CT:second:L-CT:first:L     0.119444785 -1.3735534  1.61244301 1.0000000
NH:second:L-CT:first:L     0.965345357 -0.5276529  2.45834358 0.6528733
```



```
BSA:third:L-CT:first:L      0.235260726 -1.2577375  1.72825895 1.0000000
CT:third:L-CT:first:L      -0.109875220 -1.6028734  1.38312301 1.0000000
NH:third:L-CT:first:L       0.002746498 -1.4902517  1.49574472 1.0000000
BSA:second:L-NH:first:L    -0.410003305 -1.9030015  1.08299492 0.9999155
CT:second:L-NH:first:L     -0.863214201 -2.3562124  0.62978402 0.8115716
NH:second:L-NH:first:L     -0.017313630 -1.5103119  1.47568459 1.0000000
BSA:third:L-NH:first:L     -0.747398261 -2.2403965  0.74559996 0.9324859
CT:third:L-NH:first:L      -1.092534207 -2.5855324  0.40046402 0.4341912
NH:third:L-NH:first:L      -0.979912488 -2.4729107  0.51308574 0.6279136
CT:second:L-BSA:second:L   -0.453210897 -1.9462091  1.03978733 0.9996808
NH:second:L-BSA:second:L    0.392689675 -1.1003086  1.88568790 0.9999532
BSA:third:L-BSA:second:L   -0.337394956 -1.8303932  1.15560327 0.9999946
CT:third:L-BSA:second:L    -0.682530902 -2.1755291  0.81046732 0.9690241
NH:third:L-BSA:second:L    -0.569909184 -2.0629074  0.92308904 0.9949863
NH:second:L-CT:second:L     0.845900571 -0.6470977  2.33889880 0.8342473
BSA:third:L-CT:second:L     0.115815941 -1.3771823  1.60881417 1.0000000
CT:third:L-CT:second:L     -0.229320005 -1.7223182  1.26367822 1.0000000
NH:third:L-CT:second:L     -0.116698287 -1.6096965  1.37629994 1.0000000
BSA:third:L-NH:second:L    -0.730084631 -2.2230829  0.76291359 0.9442616
CT:third:L-NH:second:L     -1.075220577 -2.5682188  0.41777765 0.4630742
NH:third:L-NH:second:L     -0.962598858 -2.4555971  0.53039937 0.6575410
CT:third:L-BSA:third:L     -0.345135946 -1.8381342  1.14786228 0.9999925
NH:third:L-BSA:third:L     -0.232514228 -1.7255125  1.26048400 1.0000000
NH:third:L-CT:third:L       0.112621718 -1.3803765  1.60561994 1.0000000
```

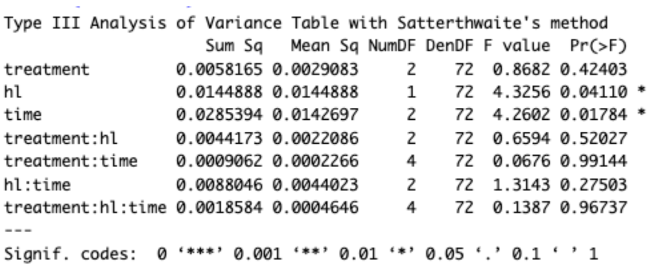
Figure 20. Tukey test of MnP activity in Hypholoma, interactions time, medium and concentration.

Hypholoma peptidase



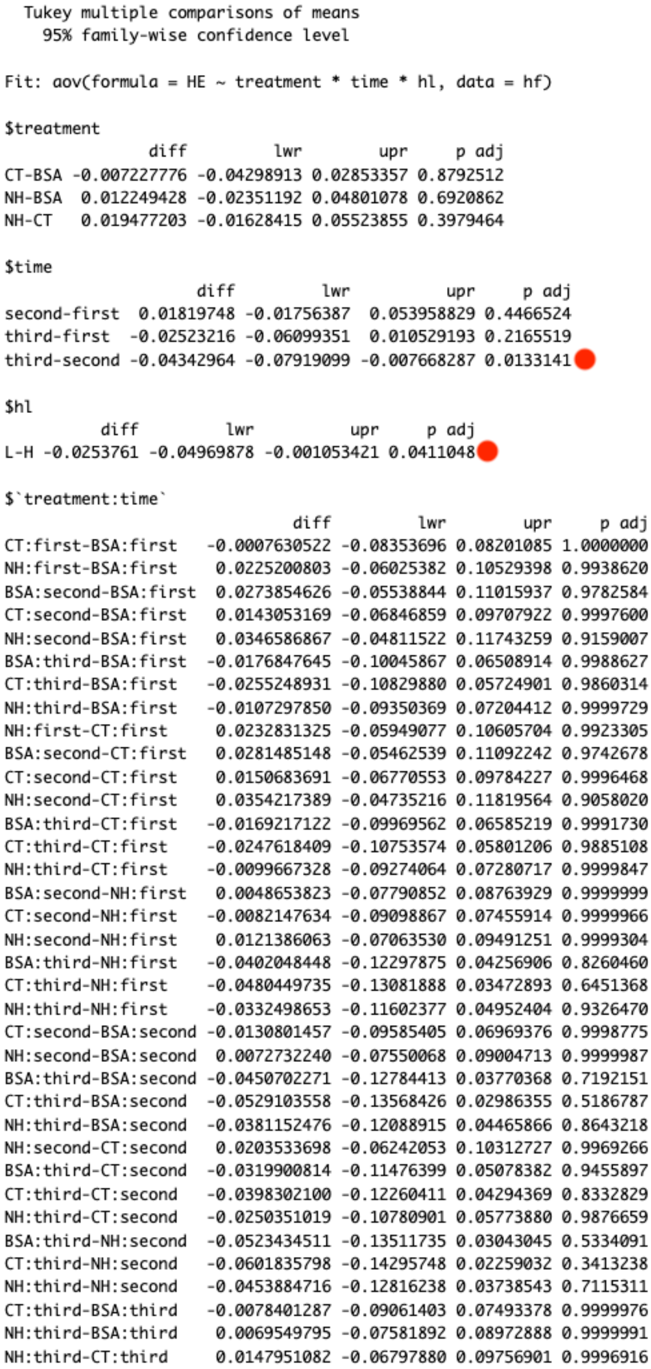
```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: HE ~ treatment * hl * time + (1 | ID)
   Data: hf

REML criterion at convergence: -177

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-1.4807 -0.3935 -0.0413  0.1240  3.7383 

Random effects:
 Groups   Name        Variance Std.Dev.
 ID       (Intercept) 0.00000  0.00000 
 Residual             0.00335  0.05788 
Number of obs: 90, groups:  ID, 30

Fixed effects:
                            Estimate Std. Error        df t value Pr(>|t|)  
(Intercept)                 0.056260   0.025883 72.000000   2.174    0.033 *
treatmentCT                -0.010858   0.036604 72.000000  -0.297    0.768  
treatmentNH                 0.022264   0.036604 72.000000   0.608    0.545  
hlL                        -0.056260   0.036604 72.000000  -1.537    0.129  
timesecond                  0.029438   0.036604 72.000000   0.804    0.424  
timethird                  -0.037759   0.036604 72.000000  -1.032    0.306  
treatmentCT:hlL             0.020191   0.051765 72.000000   0.390    0.698  
treatmentNH:hlL             0.000512   0.051765 72.000000   0.010    0.992  
treatmentCT:timesecond     -0.031779   0.051765 72.000000  -0.614    0.541  
treatmentNH:timesecond     -0.037193   0.051765 72.000000  -0.718    0.475  
treatmentCT:timethird      -0.007643   0.051765 72.000000  -0.148    0.883  
treatmentNH:timethird      -0.027450   0.051765 72.000000  -0.530    0.598  
hlL:timesecond             -0.004104   0.051765 72.000000  -0.079    0.937  
hlL:timethird               0.040148   0.051765 72.000000   0.776    0.441  
treatmentCT:hlL:timesecond  0.038924   0.073207 72.000000   0.532    0.597  
treatmentNH:hlL:timesecond  0.043892   0.073207 72.000000   0.600    0.551  
treatmentCT:hlL:timethird   0.001132   0.073207 72.000000   0.015    0.988  
treatmentNH:hlL:timethird   0.023769   0.073207 72.000000   0.325    0.746  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation matrix not shown by default, as p = 18 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it

optimizer (nloptwrap) convergence code: 0 (OK)
boundary (singular) fit: see help('isSingular')
```

Figure 21. Linear mixed model of peptidase activity in Hypholoma, interactions time, medium and concentration.

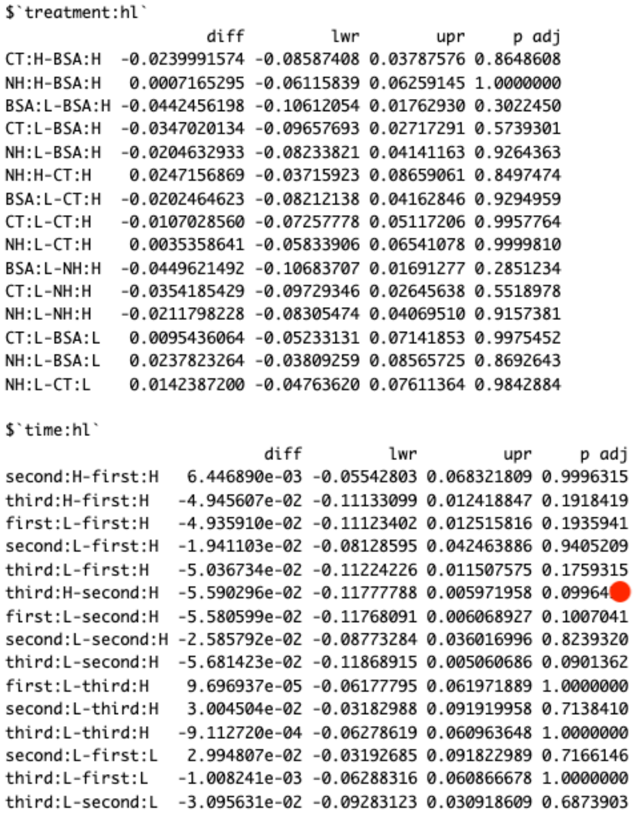


```
Type III Analysis of Variance Table with Satterthwaite's method
                      Sum Sq   Mean Sq NumDF DenDF F value  Pr(>F)  
treatment          0.0058165 0.0029083     2    72  0.8682 0.42403  
hl                 0.0144888 0.0144888     1    72  4.3256 0.04110 *
time               0.0285394 0.0142697     2    72  4.2602 0.01784 *
treatment:hl       0.0044173 0.0022086     2    72  0.6594 0.52027  
treatment:time     0.0009062 0.0002266     4    72  0.0676 0.99144  
hl:time            0.0088046 0.0044023     2    72  1.3143 0.27503  
treatment:hl:time  0.0018584 0.0004646     4    72  0.1387 0.96737  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 22. ANOVA of peptidase activity in Hypholoma, interactions time, medium and concentration.



```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = HE ~ treatment * time * hl, data = hf)

$treatment
               diff         lwr        upr     p adj
CT-BSA -0.007227776 -0.04298913 0.02853357 0.8792512
NH-BSA  0.012249428 -0.02351192 0.04801078 0.6920862
NH-CT   0.019477203 -0.01628415 0.05523855 0.3979464

$time
                    diff         lwr          upr     p adj
second-first  0.01819748 -0.01756387  0.053958829 0.4466524
third-first  -0.02523216 -0.06099351  0.010529193 0.2165519
third-second -0.04342964 -0.07919099 -0.007668287 0.0133141●

$hl
          diff         lwr          upr     p adj
L-H -0.0253761 -0.04969878 -0.001053421 0.0411048●

$`treatment:time`
                                diff         lwr        upr     p adj
CT:first-BSA:first   -0.0007630522 -0.08353696 0.08201085 1.0000000
NH:first-BSA:first    0.0225200803 -0.06025382 0.10529398 0.9938620
BSA:second-BSA:first  0.0273854626 -0.05538844 0.11015937 0.9782584
CT:second-BSA:first   0.0143053169 -0.06846859 0.09707922 0.9997600
NH:second-BSA:first   0.0346586867 -0.04811522 0.11743259 0.9159007
BSA:third-BSA:first  -0.0176847645 -0.10045867 0.06508914 0.9988627
CT:third-BSA:first   -0.0255248931 -0.10829880 0.05724901 0.9860314
NH:third-BSA:first   -0.0107297850 -0.09350369 0.07204412 0.9999729
NH:first-CT:first     0.0232831325 -0.05949077 0.10605704 0.9923305
BSA:second-CT:first   0.0281485148 -0.05462539 0.11092242 0.9742678
CT:second-CT:first    0.0150683691 -0.06770553 0.09784227 0.9996468
NH:second-CT:first    0.0354217389 -0.04735216 0.11819564 0.9058020
BSA:third-CT:first   -0.0169217122 -0.09969562 0.06585219 0.9991730
CT:third-CT:first    -0.0247618409 -0.10753574 0.05801206 0.9885108
NH:third-CT:first    -0.0099667328 -0.09274064 0.07280717 0.9999847
BSA:second-NH:first   0.0048653823 -0.07790852 0.08763929 0.9999999
CT:second-NH:first   -0.0082147634 -0.09098867 0.07455914 0.9999966
NH:second-NH:first    0.0121386063 -0.07063530 0.09491251 0.9999304
BSA:third-NH:first   -0.0402048448 -0.12297875 0.04256906 0.8260460
CT:third-NH:first    -0.0480449735 -0.13081888 0.03472893 0.6451368
NH:third-NH:first    -0.0332498653 -0.11602377 0.04952404 0.9326470
CT:second-BSA:second -0.0130801457 -0.09585405 0.06969376 0.9998775
NH:second-BSA:second  0.0072732240 -0.07550068 0.09004713 0.9999987
BSA:third-BSA:second -0.0450702271 -0.12784413 0.03770368 0.7192151
CT:third-BSA:second  -0.0529103558 -0.13568426 0.02986355 0.5186787
NH:third-BSA:second  -0.0381152476 -0.12088915 0.04465866 0.8643218
NH:second-CT:second   0.0203533698 -0.06242053 0.10312727 0.9969266
BSA:third-CT:second  -0.0319900814 -0.11476399 0.05078382 0.9455897
CT:third-CT:second   -0.0398302100 -0.12260411 0.04294369 0.8332829
NH:third-CT:second   -0.0250351019 -0.10780901 0.05773880 0.9876659
BSA:third-NH:second  -0.0523434511 -0.13511735 0.03043045 0.5334091
CT:third-NH:second   -0.0601835798 -0.14295748 0.02259032 0.3413238
NH:third-NH:second   -0.0453884716 -0.12816238 0.03738543 0.7115311
CT:third-BSA:third   -0.0078401287 -0.09061403 0.07493378 0.9999976
NH:third-BSA:third    0.0069549795 -0.07581892 0.08972888 0.9999991
NH:third-CT:third     0.0147951082 -0.06797880 0.09756901 0.9996916
```



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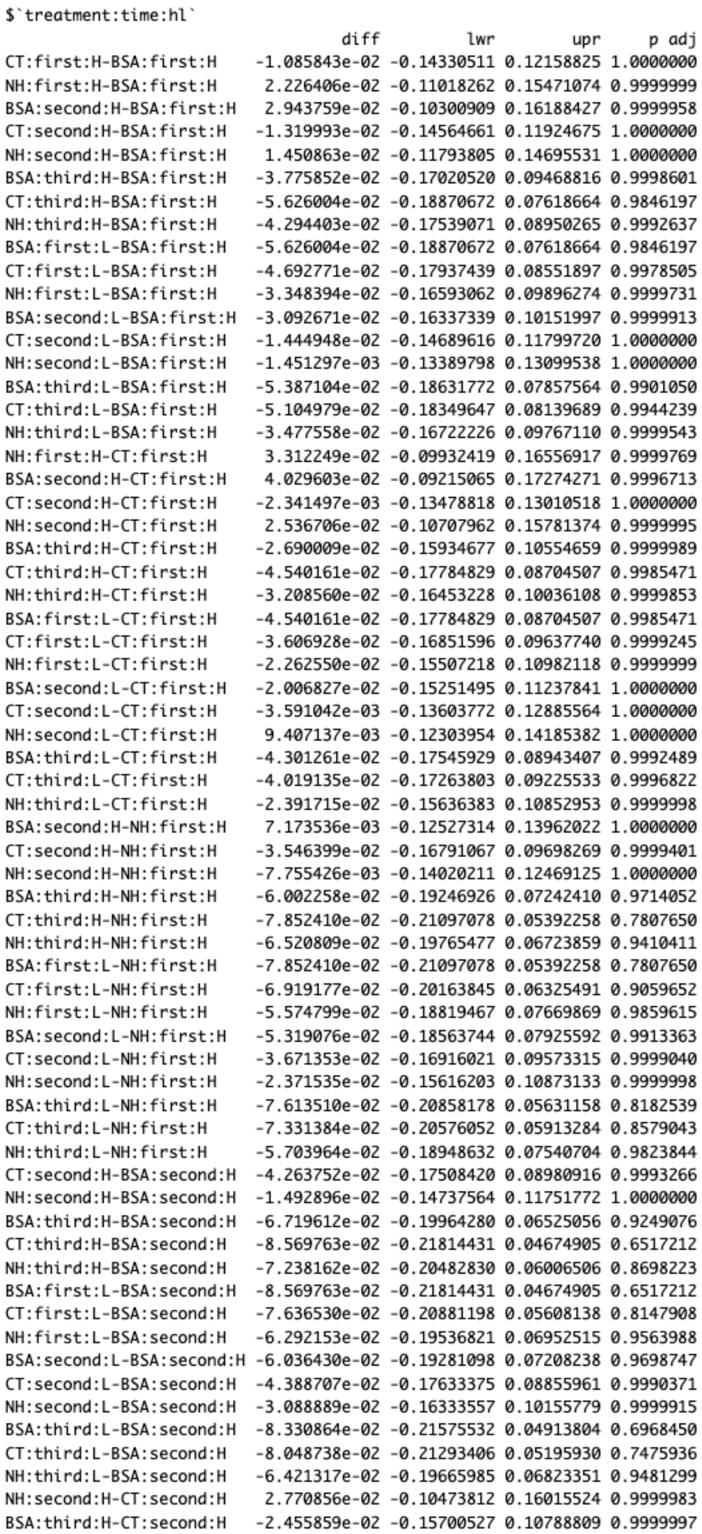
$`treatment:hl`
                    diff         lwr        upr     p adj
CT:H-BSA:H  -0.0239991574 -0.08587408 0.03787576 0.8648608
NH:H-BSA:H   0.0007165295 -0.06115839 0.06259145 1.0000000
BSA:L-BSA:H -0.0442456198 -0.10612054 0.01762930 0.3022450
CT:L-BSA:H  -0.0347020134 -0.09657693 0.02717291 0.5739301
NH:L-BSA:H  -0.0204632933 -0.08233821 0.04141163 0.9264363
NH:H-CT:H    0.0247156869 -0.03715923 0.08659061 0.8497474
BSA:L-CT:H  -0.0202464623 -0.08212138 0.04162846 0.9294959
CT:L-CT:H   -0.0107028560 -0.07257778 0.05117206 0.9957764
NH:L-CT:H    0.0035358641 -0.05833906 0.06541078 0.9999810
BSA:L-NH:H  -0.0449621492 -0.10683707 0.01691277 0.2851234
CT:L-NH:H   -0.0354185429 -0.09729346 0.02645638 0.5518978
NH:L-NH:H   -0.0211798228 -0.08305474 0.04069510 0.9157381
CT:L-BSA:L   0.0095436064 -0.05233131 0.07141853 0.9975452
NH:L-BSA:L   0.0237823264 -0.03809259 0.08565725 0.8692643
NH:L-CT:L    0.0142387200 -0.04763620 0.07611364 0.9842884

$`time:hl`
                          diff         lwr         upr     p adj
second:H-first:H   6.446890e-03 -0.05542803 0.068321809 0.9996315
third:H-first:H   -4.945607e-02 -0.11133099 0.012418847 0.1918419
first:L-first:H   -4.935910e-02 -0.11123402 0.012515816 0.1935941
second:L-first:H  -1.941103e-02 -0.08128595 0.042463886 0.9405209
third:L-first:H   -5.036734e-02 -0.11224226 0.011507575 0.1759315
third:H-second:H  -5.590296e-02 -0.11777788 0.005971958 0.09964
first:L-second:H  -5.580599e-02 -0.11768091 0.006068927 0.1007041
second:L-second:H -2.585792e-02 -0.08773284 0.036016996 0.8239320
third:L-second:H  -5.681423e-02 -0.11868915 0.005060686 0.0901362
first:L-third:H    9.696937e-05 -0.06177795 0.061971889 1.0000000
second:L-third:H   3.004504e-02 -0.03182988 0.091919958 0.7138410
third:L-third:H   -9.112720e-04 -0.06278619 0.060963648 1.0000000
second:L-first:L   2.994807e-02 -0.03192685 0.091822989 0.7166146
third:L-first:L   -1.008241e-03 -0.06288316 0.060866678 1.0000000
third:L-second:L  -3.095631e-02 -0.09283123 0.030918609 0.6873903
```



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NH:first:H-BSA:first:H     2.226406e-02 -0.11018262 0.15471074 0.9999999
BSA:second:H-BSA:first:H   2.943759e-02 -0.10300909 0.16188427 0.9999958
CT:second:H-BSA:first:H   -1.319993e-02 -0.14564661 0.11924675 1.0000000
NH:second:H-BSA:first:H    1.450863e-02 -0.11793805 0.14695531 1.0000000
BSA:third:H-BSA:first:H   -3.775852e-02 -0.17020520 0.09468816 0.9998601
CT:third:H-BSA:first:H    -5.626004e-02 -0.18870672 0.07618664 0.9846197
NH:third:H-BSA:first:H    -4.294403e-02 -0.17539071 0.08950265 0.9992637
BSA:first:L-BSA:first:H   -5.626004e-02 -0.18870672 0.07618664 0.9846197
CT:first:L-BSA:first:H    -4.692771e-02 -0.17937439 0.08551897 0.9978505
NH:first:L-BSA:first:H    -3.348394e-02 -0.16593062 0.09896274 0.9999731
BSA:second:L-BSA:first:H  -3.092671e-02 -0.16337339 0.10151997 0.9999913
CT:second:L-BSA:first:H   -1.444948e-02 -0.14689616 0.11799720 1.0000000
NH:second:L-BSA:first:H   -1.451297e-03 -0.13389798 0.13099538 1.0000000
BSA:third:L-BSA:first:H   -5.387104e-02 -0.18631772 0.07857564 0.9901050
CT:third:L-BSA:first:H    -5.104979e-02 -0.18349647 0.08139689 0.9944239
NH:third:L-BSA:first:H    -3.477558e-02 -0.16722226 0.09767110 0.9999543
NH:first:H-CT:first:H      3.312249e-02 -0.09932419 0.16556917 0.9999769
BSA:second:H-CT:first:H    4.029603e-02 -0.09215065 0.17274271 0.9996713
CT:second:H-CT:first:H    -2.341497e-03 -0.13478818 0.13010518 1.0000000
NH:second:H-CT:first:H     2.536706e-02 -0.10707962 0.15781374 0.9999995
BSA:third:H-CT:first:H    -2.690009e-02 -0.15934677 0.10554659 0.9999989
CT:third:H-CT:first:H     -4.540161e-02 -0.17784829 0.08704507 0.9985471
NH:third:H-CT:first:H     -3.208560e-02 -0.16453228 0.10036108 0.9999853
BSA:first:L-CT:first:H    -4.540161e-02 -0.17784829 0.08704507 0.9985471
CT:first:L-CT:first:H     -3.606928e-02 -0.16851596 0.09637740 0.9999245
NH:first:L-CT:first:H     -2.262550e-02 -0.15507218 0.10982118 0.9999999
BSA:second:L-CT:first:H   -2.006827e-02 -0.15251495 0.11237841 1.0000000
CT:second:L-CT:first:H    -3.591042e-03 -0.13603772 0.12885564 1.0000000
NH:second:L-CT:first:H     9.407137e-03 -0.12303954 0.14185382 1.0000000
BSA:third:L-CT:first:H    -4.301261e-02 -0.17545929 0.08943407 0.9992489
CT:third:L-CT:first:H     -4.019135e-02 -0.17263803 0.09225533 0.9996822
NH:third:L-CT:first:H     -2.391715e-02 -0.15636383 0.10852953 0.9999998
BSA:second:H-NH:first:H    7.173536e-03 -0.12527314 0.13962022 1.0000000
CT:second:H-NH:first:H    -3.546399e-02 -0.16791067 0.09698269 0.9999401
NH:second:H-NH:first:H    -7.755426e-03 -0.14020211 0.12469125 1.0000000
BSA:third:H-NH:first:H    -6.002258e-02 -0.19246926 0.07242410 0.9714052
CT:third:H-NH:first:H     -7.852410e-02 -0.21097078 0.05392258 0.7807650
NH:third:H-NH:first:H     -6.520809e-02 -0.19765477 0.06723859 0.9410411
BSA:first:L-NH:first:H    -7.852410e-02 -0.21097078 0.05392258 0.7807650
CT:first:L-NH:first:H     -6.919177e-02 -0.20163845 0.06325491 0.9059652
NH:first:L-NH:first:H     -5.574799e-02 -0.18819467 0.07669869 0.9859615
BSA:second:L-NH:first:H   -5.319076e-02 -0.18563744 0.07925592 0.9913363
CT:second:L-NH:first:H    -3.671353e-02 -0.16916021 0.09573315 0.9999040
NH:second:L-NH:first:H    -2.371535e-02 -0.15616203 0.10873133 0.9999998
BSA:third:L-NH:first:H    -7.613510e-02 -0.20858178 0.05631158 0.8182539
CT:third:L-NH:first:H     -7.331384e-02 -0.20576052 0.05913284 0.8579043
NH:third:L-NH:first:H     -5.703964e-02 -0.18948632 0.07540704 0.9823844
CT:second:H-BSA:second:H  -4.263752e-02 -0.17508420 0.08980916 0.9993266
NH:second:H-BSA:second:H  -1.492896e-02 -0.14737564 0.11751772 1.0000000
BSA:third:H-BSA:second:H  -6.719612e-02 -0.19964280 0.06525056 0.9249076
CT:third:H-BSA:second:H   -8.569763e-02 -0.21814431 0.04674905 0.6517212
NH:third:H-BSA:second:H   -7.238162e-02 -0.20482830 0.06006506 0.8698223
BSA:first:L-BSA:second:H  -8.569763e-02 -0.21814431 0.04674905 0.6517212
CT:first:L-BSA:second:H   -7.636530e-02 -0.20881198 0.05608138 0.8147908
NH:first:L-BSA:second:H   -6.292153e-02 -0.19536821 0.06952515 0.9563988
BSA:second:L-BSA:second:H -6.036430e-02 -0.19281098 0.07208238 0.9698747
CT:second:L-BSA:second:H  -4.388707e-02 -0.17633375 0.08855961 0.9990371
NH:second:L-BSA:second:H  -3.088889e-02 -0.16333557 0.10155779 0.9999915
BSA:third:L-BSA:second:H  -8.330864e-02 -0.21575532 0.04913804 0.6968450
CT:third:L-BSA:second:H   -8.048738e-02 -0.21293406 0.05195930 0.7475936
NH:third:L-BSA:second:H   -6.421317e-02 -0.19665985 0.06823351 0.9481299
NH:second:H-CT:second:H    2.770856e-02 -0.10473812 0.16015524 0.9999983
BSA:third:H-CT:second:H   -2.455859e-02 -0.15700527 0.10788809 0.9999997
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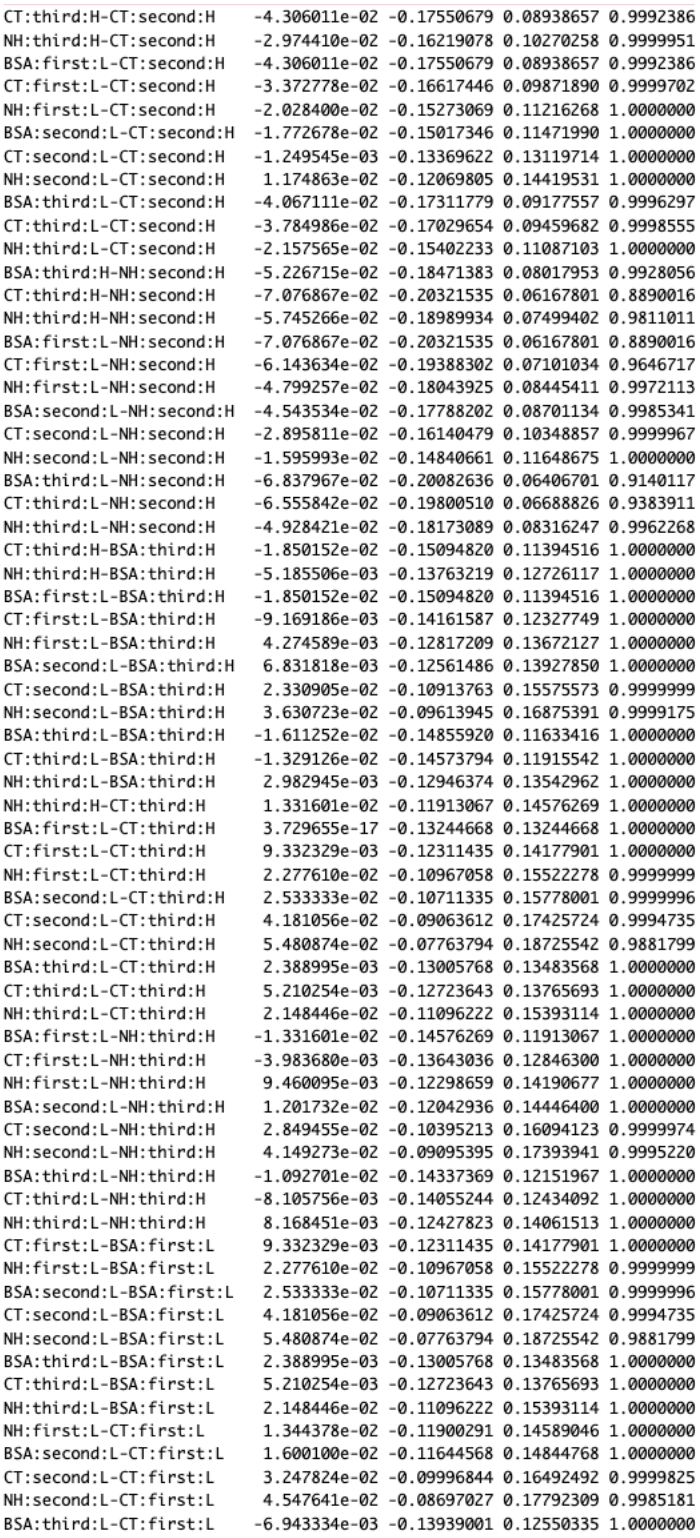

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NH:third:H-CT:second:H    -2.974410e-02 -0.16219078 0.10270258 0.9999951
BSA:first:L-CT:second:H   -4.306011e-02 -0.17550679 0.08938657 0.9992386
CT:first:L-CT:second:H    -3.372778e-02 -0.16617446 0.09871890 0.9999702
NH:first:L-CT:second:H    -2.028400e-02 -0.15273069 0.11216268 1.0000000
BSA:second:L-CT:second:H  -1.772678e-02 -0.15017346 0.11471990 1.0000000
CT:second:L-CT:second:H   -1.249545e-03 -0.13369622 0.13119714 1.0000000
NH:second:L-CT:second:H    1.174863e-02 -0.12069805 0.14419531 1.0000000
BSA:third:L-CT:second:H   -4.067111e-02 -0.17311779 0.09177557 0.9996297
CT:third:L-CT:second:H    -3.784986e-02 -0.17029654 0.09459682 0.9998555
NH:third:L-CT:second:H    -2.157565e-02 -0.15402233 0.11087103 1.0000000
BSA:third:H-NH:second:H   -5.226715e-02 -0.18471383 0.08017953 0.9928056
CT:third:H-NH:second:H    -7.076867e-02 -0.20321535 0.06167801 0.8890016
NH:third:H-NH:second:H    -5.745266e-02 -0.18989934 0.07499402 0.9811011
BSA:first:L-NH:second:H   -7.076867e-02 -0.20321535 0.06167801 0.8890016
CT:first:L-NH:second:H    -6.143634e-02 -0.19388302 0.07101034 0.9646717
NH:first:L-NH:second:H    -4.799257e-02 -0.18043925 0.08445411 0.9972113
BSA:second:L-NH:second:H  -4.543534e-02 -0.17788202 0.08701134 0.9985341
CT:second:L-NH:second:H   -2.895811e-02 -0.16140479 0.10348857 0.9999967
NH:second:L-NH:second:H   -1.595993e-02 -0.14840661 0.11648675 1.0000000
BSA:third:L-NH:second:H   -6.837967e-02 -0.20082636 0.06406701 0.9140117
CT:third:L-NH:second:H    -6.555842e-02 -0.19800510 0.06688826 0.9383911
NH:third:L-NH:second:H    -4.928421e-02 -0.18173089 0.08316247 0.9962268
CT:third:H-BSA:third:H    -1.850152e-02 -0.15094820 0.11394516 1.0000000
NH:third:H-BSA:third:H    -5.185506e-03 -0.13763219 0.12726117 1.0000000
BSA:first:L-BSA:third:H   -1.850152e-02 -0.15094820 0.11394516 1.0000000
CT:first:L-BSA:third:H    -9.169186e-03 -0.14161587 0.12327749 1.0000000
NH:first:L-BSA:third:H     4.274589e-03 -0.12817209 0.13672127 1.0000000
BSA:second:L-BSA:third:H   6.831818e-03 -0.12561486 0.13927850 1.0000000
CT:second:L-BSA:third:H    2.330905e-02 -0.10913763 0.15575573 0.9999999
NH:second:L-BSA:third:H    3.630723e-02 -0.09613945 0.16875391 0.9999175
BSA:third:L-BSA:third:H   -1.611252e-02 -0.14855920 0.11633416 1.0000000
CT:third:L-BSA:third:H    -1.329126e-02 -0.14573794 0.11915542 1.0000000
NH:third:L-BSA:third:H     2.982945e-03 -0.12946374 0.13542962 1.0000000
NH:third:H-CT:third:H      1.331601e-02 -0.11913067 0.14576269 1.0000000
BSA:first:L-CT:third:H     3.729655e-17 -0.13244668 0.13244668 1.0000000
CT:first:L-CT:third:H      9.332329e-03 -0.12311435 0.14177901 1.0000000
NH:first:L-CT:third:H      2.277610e-02 -0.10967058 0.15522278 0.9999999
BSA:second:L-CT:third:H    2.533333e-02 -0.10711335 0.15778001 0.9999996
CT:second:L-CT:third:H     4.181056e-02 -0.09063612 0.17425724 0.9994735
NH:second:L-CT:third:H     5.480874e-02 -0.07763794 0.18725542 0.9881799
BSA:third:L-CT:third:H     2.388995e-03 -0.13005768 0.13483568 1.0000000
CT:third:L-CT:third:H      5.210254e-03 -0.12723643 0.13765693 1.0000000
NH:third:L-CT:third:H      2.148446e-02 -0.11096222 0.15393114 1.0000000
BSA:first:L-NH:third:H    -1.331601e-02 -0.14576269 0.11913067 1.0000000
CT:first:L-NH:third:H     -3.983680e-03 -0.13643036 0.12846300 1.0000000
NH:first:L-NH:third:H      9.460095e-03 -0.12298659 0.14190677 1.0000000
BSA:second:L-NH:third:H    1.201732e-02 -0.12042936 0.14446400 1.0000000
CT:second:L-NH:third:H     2.849455e-02 -0.10395213 0.16094123 0.9999974
NH:second:L-NH:third:H     4.149273e-02 -0.09095395 0.17393941 0.9995220
BSA:third:L-NH:third:H    -1.092701e-02 -0.14337369 0.12151967 1.0000000
CT:third:L-NH:third:H     -8.105756e-03 -0.14055244 0.12434092 1.0000000
NH:third:L-NH:third:H      8.168451e-03 -0.12427823 0.14061513 1.0000000
CT:first:L-BSA:first:L     9.332329e-03 -0.12311435 0.14177901 1.0000000
NH:first:L-BSA:first:L     2.277610e-02 -0.10967058 0.15522278 0.9999999
BSA:second:L-BSA:first:L   2.533333e-02 -0.10711335 0.15778001 0.9999996
CT:second:L-BSA:first:L    4.181056e-02 -0.09063612 0.17425724 0.9994735
NH:second:L-BSA:first:L    5.480874e-02 -0.07763794 0.18725542 0.9881799
BSA:third:L-BSA:first:L    2.388995e-03 -0.13005768 0.13483568 1.0000000
CT:third:L-BSA:first:L     5.210254e-03 -0.12723643 0.13765693 1.0000000
NH:third:L-BSA:first:L     2.148446e-02 -0.11096222 0.15393114 1.0000000
NH:first:L-CT:first:L      1.344378e-02 -0.11900291 0.14589046 1.0000000
BSA:second:L-CT:first:L    1.600100e-02 -0.11644568 0.14844768 1.0000000
CT:second:L-CT:first:L     3.247824e-02 -0.09996844 0.16492492 0.9999825
NH:second:L-CT:first:L     4.547641e-02 -0.08697027 0.17792309 0.9985181
BSA:third:L-CT:first:L    -6.943334e-03 -0.13939001 0.12550335 1.0000000
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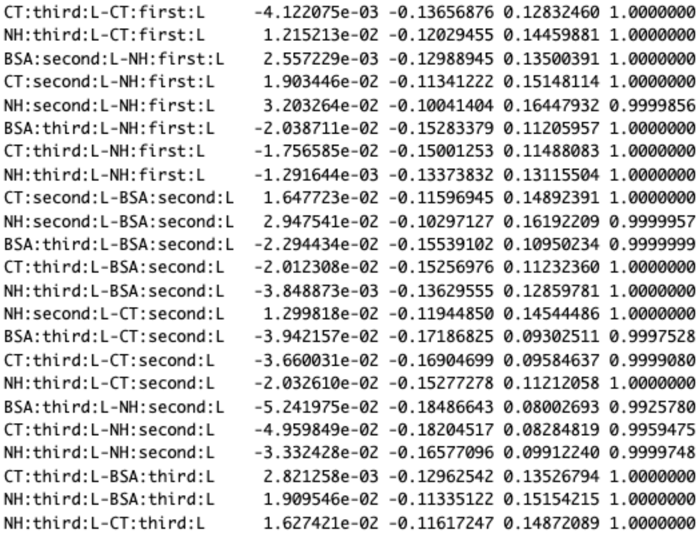

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CT:third:L-CT:first:L       -4.122075e-03 -0.13656876 0.12832460 1.0000000
NH:third:L-CT:first:L        1.215213e-02 -0.12029455 0.14459881 1.0000000
BSA:second:L-NH:first:L      2.557229e-03 -0.12988945 0.13500391 1.0000000
CT:second:L-NH:first:L       1.903446e-02 -0.11341222 0.15148114 1.0000000
NH:second:L-NH:first:L       3.203264e-02 -0.10041404 0.16447932 0.9999856
BSA:third:L-NH:first:L      -2.038711e-02 -0.15283379 0.11205957 1.0000000
CT:third:L-NH:first:L       -1.756585e-02 -0.15001253 0.11488083 1.0000000
NH:third:L-NH:first:L       -1.291644e-03 -0.13373832 0.13115504 1.0000000
CT:second:L-BSA:second:L     1.647723e-02 -0.11596945 0.14892391 1.0000000
NH:second:L-BSA:second:L     2.947541e-02 -0.10297127 0.16192209 0.9999957
BSA:third:L-BSA:second:L    -2.294434e-02 -0.15539102 0.10950234 0.9999999
CT:third:L-BSA:second:L     -2.012308e-02 -0.15256976 0.11232360 1.0000000
NH:third:L-BSA:second:L     -3.848873e-03 -0.13629555 0.12859781 1.0000000
NH:second:L-CT:second:L      1.299818e-02 -0.11944850 0.14544486 1.0000000
BSA:third:L-CT:second:L     -3.942157e-02 -0.17186825 0.09302511 0.9997528
CT:third:L-CT:second:L      -3.660031e-02 -0.16904699 0.09584637 0.9999080
NH:third:L-CT:second:L      -2.032610e-02 -0.15277278 0.11212058 1.0000000
BSA:third:L-NH:second:L     -5.241975e-02 -0.18486643 0.08002693 0.9925780
CT:third:L-NH:second:L      -4.959849e-02 -0.18204517 0.08284819 0.9959475
NH:third:L-NH:second:L      -3.332428e-02 -0.16577096 0.09912240 0.9999748
CT:third:L-BSA:third:L       2.821258e-03 -0.12962542 0.13526794 1.0000000
NH:third:L-BSA:third:L       1.909546e-02 -0.11335122 0.15154215 1.0000000
NH:third:L-CT:third:L        1.627421e-02 -0.11617247 0.14872089 1.0000000
```
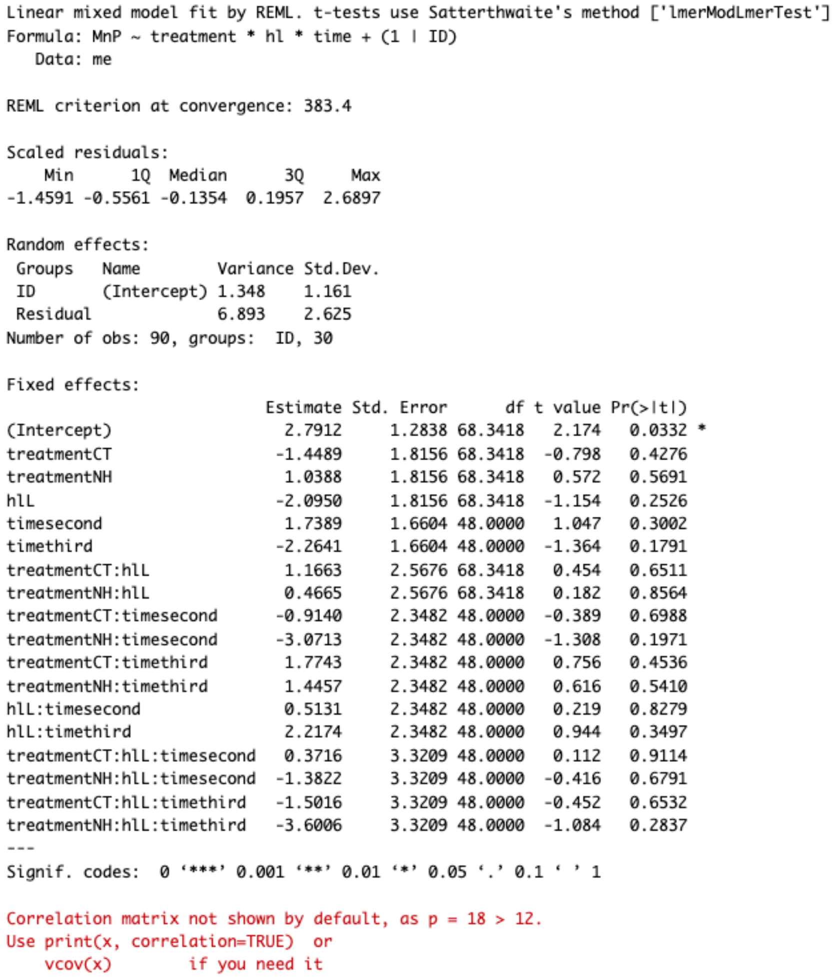
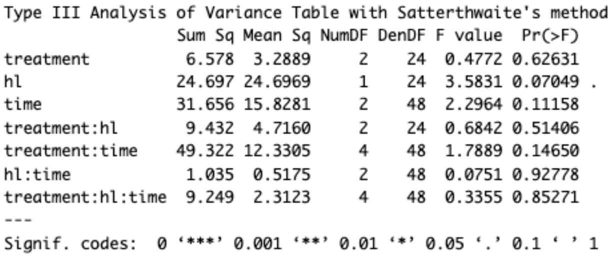

Figure 23. Tukey test of peptidase activity in Hypholoma, interactions time, medium and concentration

Mycena MnP



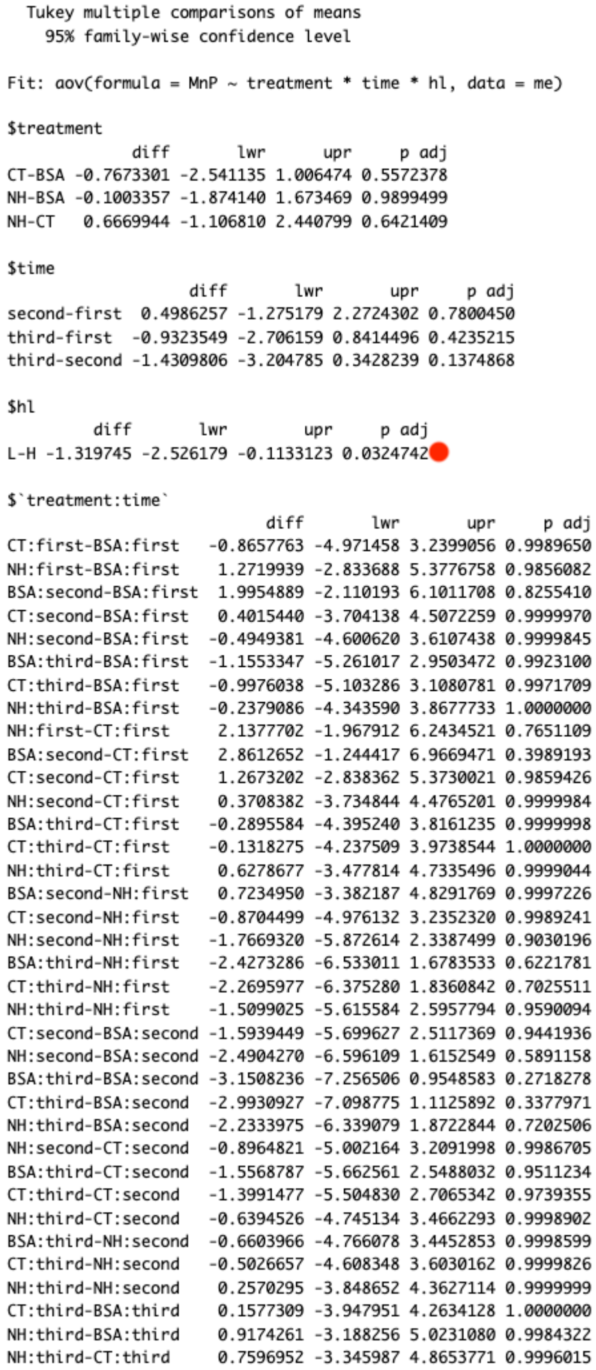
```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: MnP ~ treatment * hl * time + (1 | ID)
   Data: me

REML criterion at convergence: 383.4

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4591 -0.5561 -0.1354  0.1957  2.6897

Random effects:
 Groups   Name        Variance Std.Dev.
 ID       (Intercept) 1.348    1.161
 Residual             6.893    2.625
Number of obs: 90, groups:  ID, 30

Fixed effects:
                           Estimate Std. Error      df t value Pr(>|t|)
(Intercept)                  2.7912     1.2838 68.3418   2.174   0.0332 *
treatmentCT                 -1.4489     1.8156 68.3418  -0.798   0.4276
treatmentNH                  1.0388     1.8156 68.3418   0.572   0.5691
hlL                         -2.0950     1.8156 68.3418  -1.154   0.2526
timesecond                   1.7389     1.6604 48.0000   1.047   0.3002
timethird                   -2.2641     1.6604 48.0000  -1.364   0.1791
treatmentCT:hlL              1.1663     2.5676 68.3418   0.454   0.6511
treatmentNH:hlL              0.4665     2.5676 68.3418   0.182   0.8564
treatmentCT:timesecond      -0.9140     2.3482 48.0000  -0.389   0.6988
treatmentNH:timesecond      -3.0713     2.3482 48.0000  -1.308   0.1971
treatmentCT:timethird        1.7743     2.3482 48.0000   0.756   0.4536
treatmentNH:timethird        1.4457     2.3482 48.0000   0.616   0.5410
hlL:timesecond               0.5131     2.3482 48.0000   0.219   0.8279
hlL:timethird                2.2174     2.3482 48.0000   0.944   0.3497
treatmentCT:hlL:timesecond   0.3716     3.3209 48.0000   0.112   0.9114
treatmentNH:hlL:timesecond  -1.3822     3.3209 48.0000  -0.416   0.6791
treatmentCT:hlL:timethird   -1.5016     3.3209 48.0000  -0.452   0.6532
treatmentNH:hlL:timethird   -3.6006     3.3209 48.0000  -1.084   0.2837
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation matrix not shown by default, as p = 18 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it
```

Figure 24. Linear mixed model of MnP activity in Mycena, interactions time, medium and concentration.

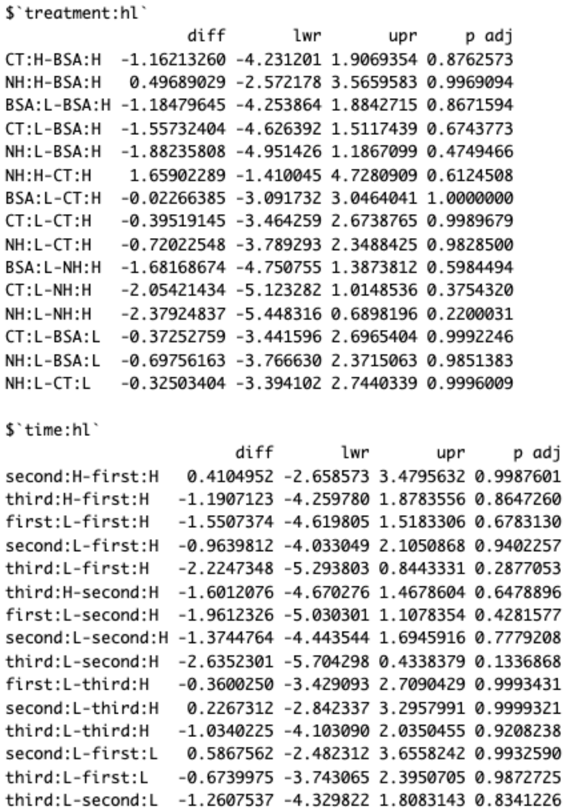


```
Type III Analysis of Variance Table with Satterthwaite's method
                   Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
treatment           6.578  3.2889     2    24  0.4772 0.62631
hl                 24.697 24.6969     1    24  3.5831 0.07049 .
time               31.656 15.8281     2    48  2.2964 0.11158
treatment:hl        9.432  4.7160     2    24  0.6842 0.51406
treatment:time     49.322 12.3305     4    48  1.7889 0.14650
hl:time             1.035  0.5175     2    48  0.0751 0.92778
treatment:hl:time   9.249  2.3123     4    48  0.3355 0.85271
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

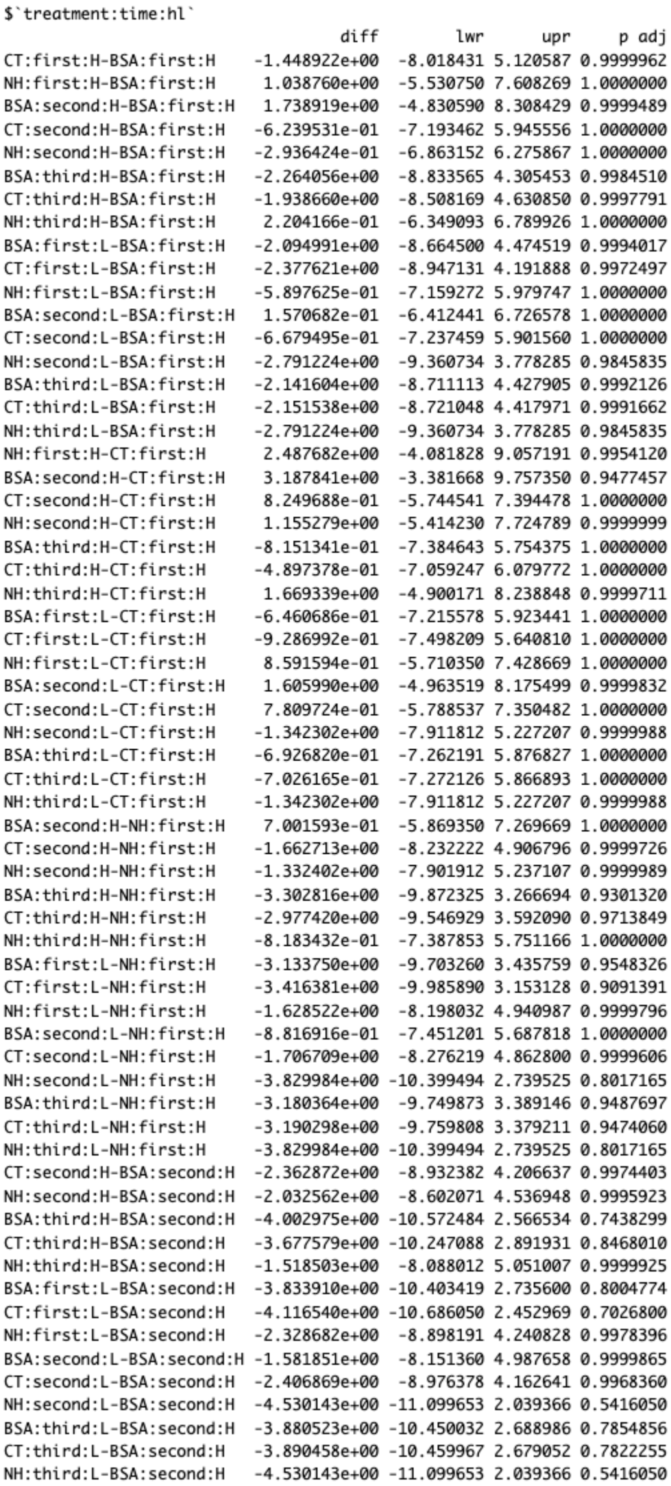
Figure 25. ANOVA of MnP activity in Mycena, interactions time, medium and concentration.



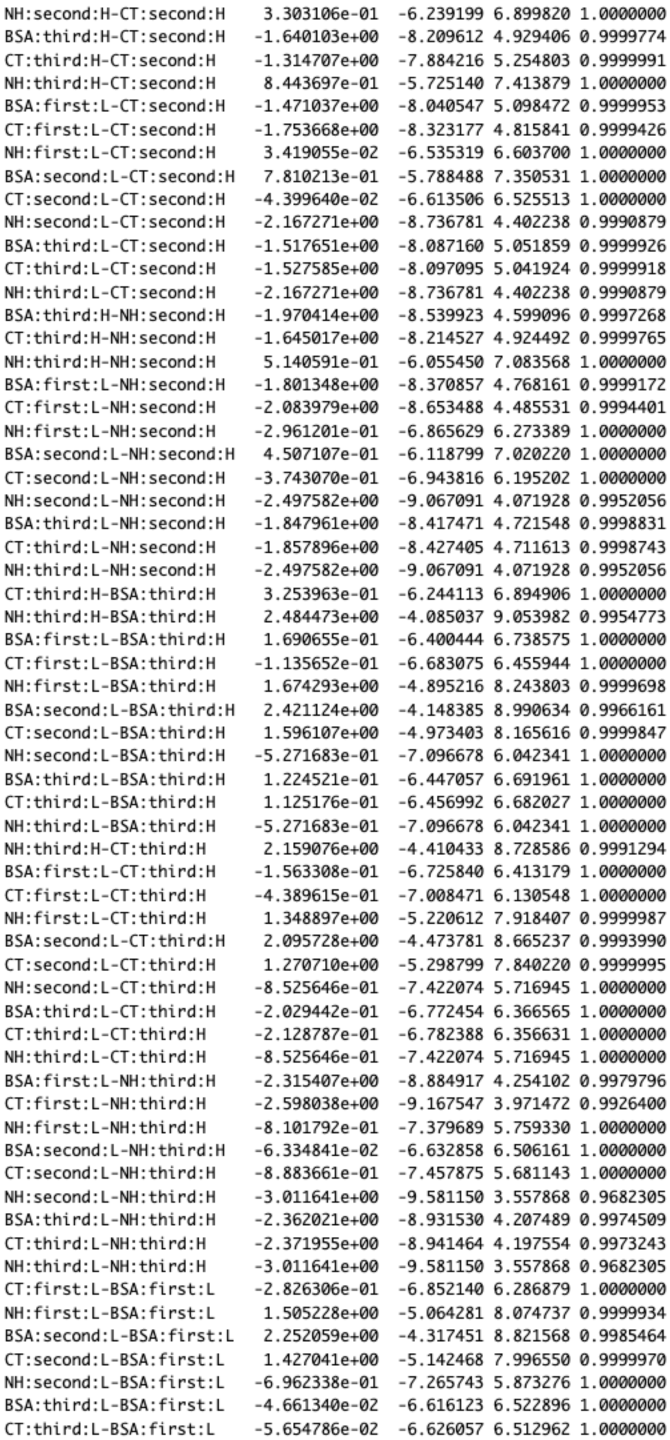
```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = MnP ~ treatment * time * hl, data = me)

$treatment
             diff       lwr      upr     p adj
CT-BSA -0.7673301 -2.541135 1.006474 0.5572378
NH-BSA -0.1003357 -1.874140 1.673469 0.9899499
NH-CT   0.6669944 -1.106810 2.440799 0.6421409

$time
                   diff       lwr       upr     p adj
second-first  0.4986257 -1.275179 2.2724302 0.7800450
third-first  -0.9323549 -2.706159 0.8414496 0.4235215
third-second -1.4309806 -3.204785 0.3428239 0.1374868

$hl
         diff       lwr        upr     p adj
L-H -1.319745 -2.526179 -0.1133123 0.0324742●

$`treatment:time`
                           diff       lwr       upr     p adj
CT:first-BSA:first   -0.8657763 -4.971458 3.2399056 0.9989650
NH:first-BSA:first    1.2719939 -2.833688 5.3776758 0.9856082
BSA:second-BSA:first  1.9954889 -2.110193 6.1011708 0.8255410
CT:second-BSA:first   0.4015440 -3.704138 4.5072259 0.9999970
NH:second-BSA:first  -0.4949381 -4.600620 3.6107438 0.9999845
BSA:third-BSA:first  -1.1553347 -5.261017 2.9503472 0.9923100
CT:third-BSA:first   -0.9976038 -5.103286 3.1080781 0.9971709
NH:third-BSA:first   -0.2379086 -4.343590 3.8677733 1.0000000
NH:first-CT:first     2.1377702 -1.967912 6.2434521 0.7651109
BSA:second-CT:first   2.8612652 -1.244417 6.9669471 0.3989193
CT:second-CT:first    1.2673202 -2.838362 5.3730021 0.9859426
NH:second-CT:first    0.3708382 -3.734844 4.4765201 0.9999984
BSA:third-CT:first   -0.2895584 -4.395240 3.8161235 0.9999998
CT:third-CT:first    -0.1318275 -4.237509 3.9738544 1.0000000
NH:third-CT:first     0.6278677 -3.477814 4.7335496 0.9999044
BSA:second-NH:first   0.7234950 -3.382187 4.8291769 0.9997226
CT:second-NH:first   -0.8704499 -4.976132 3.2352320 0.9989241
NH:second-NH:first   -1.7669320 -5.872614 2.3387499 0.9030196
BSA:third-NH:first   -2.4273286 -6.533011 1.6783533 0.6221781
CT:third-NH:first    -2.2695977 -6.375280 1.8360842 0.7025511
NH:third-NH:first    -1.5099025 -5.615584 2.5957794 0.9590094
CT:second-BSA:second -1.5939449 -5.699627 2.5117369 0.9441936
NH:second-BSA:second -2.4904270 -6.596109 1.6152549 0.5891158
BSA:third-BSA:second -3.1508236 -7.256506 0.9548583 0.2718278
CT:third-BSA:second  -2.9930927 -7.098775 1.1125892 0.3377971
NH:third-BSA:second  -2.2333975 -6.339079 1.8722844 0.7202506
NH:second-CT:second  -0.8964821 -5.002164 3.2091998 0.9986705
BSA:third-CT:second  -1.5568787 -5.662561 2.5488032 0.9511234
CT:third-CT:second   -1.3991477 -5.504830 2.7065342 0.9739355
NH:third-CT:second   -0.6394526 -4.745134 3.4662293 0.9998902
BSA:third-NH:second  -0.6603966 -4.766078 3.4452853 0.9998599
CT:third-NH:second   -0.5026657 -4.608348 3.6030162 0.9999826
NH:third-NH:second    0.2570295 -3.848652 4.3627114 0.9999999
CT:third-BSA:third    0.1577309 -3.947951 4.2634128 1.0000000
NH:third-BSA:third    0.9174261 -3.188256 5.0231080 0.9984322
NH:third-CT:third     0.7596952 -3.345987 4.8653771 0.9996015
```



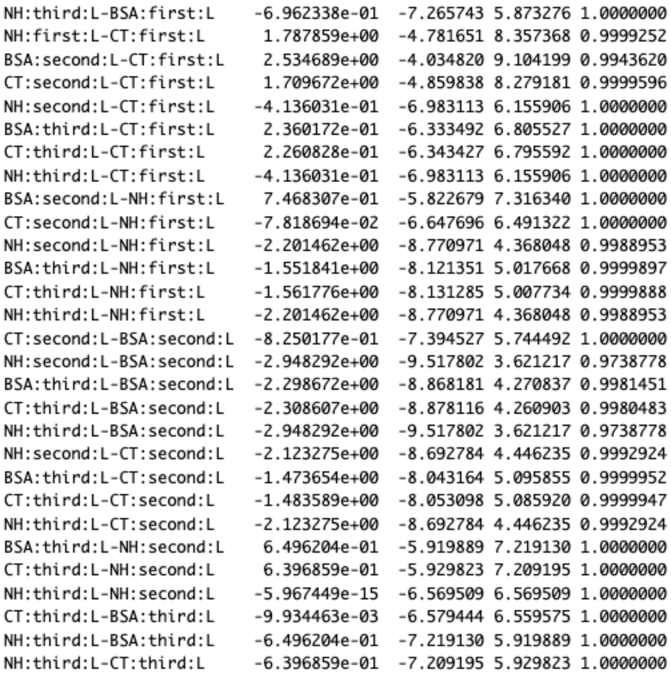
```
$`treatment:hl`
                   diff       lwr       upr     p adj
CT:H-BSA:H  -1.16213260 -4.231201 1.9069354 0.8762573
NH:H-BSA:H   0.49689029 -2.572178 3.5659583 0.9969094
BSA:L-BSA:H -1.18479645 -4.253864 1.8842715 0.8671594
CT:L-BSA:H  -1.55732404 -4.626392 1.5117439 0.6743773
NH:L-BSA:H  -1.88235808 -4.951426 1.1867099 0.4749466
NH:H-CT:H    1.65902289 -1.410045 4.7280909 0.6124508
BSA:L-CT:H  -0.02266385 -3.091732 3.0464041 1.0000000
CT:L-CT:H   -0.39519145 -3.464259 2.6738765 0.9989679
NH:L-CT:H   -0.72022548 -3.789293 2.3488425 0.9828500
BSA:L-NH:H  -1.68168674 -4.750755 1.3873812 0.5984494
CT:L-NH:H   -2.05421434 -5.123282 1.0148536 0.3754320
NH:L-NH:H   -2.37924837 -5.448316 0.6898196 0.2200031
CT:L-BSA:L  -0.37252759 -3.441596 2.6965404 0.9992246
NH:L-BSA:L  -0.69756163 -3.766630 2.3715063 0.9851383
NH:L-CT:L   -0.32503404 -3.394102 2.7440339 0.9996009

$`time:hl`
                        diff       lwr       upr     p adj
second:H-first:H   0.4104952 -2.658573 3.4795632 0.9987601
third:H-first:H   -1.1907123 -4.259780 1.8783556 0.8647260
first:L-first:H   -1.5507374 -4.619805 1.5183306 0.6783130
second:L-first:H  -0.9639812 -4.033049 2.1050868 0.9402257
third:L-first:H   -2.2247348 -5.293803 0.8443331 0.2877053
third:H-second:H  -1.6012076 -4.670276 1.4678604 0.6478896
first:L-second:H  -1.9612326 -5.030301 1.1078354 0.4281577
second:L-second:H -1.3744764 -4.443544 1.6945916 0.7779208
third:L-second:H  -2.6352301 -5.704298 0.4338379 0.1336868
first:L-third:H   -0.3600250 -3.429093 2.7090429 0.9993431
second:L-third:H   0.2267312 -2.842337 3.2957991 0.9999321
third:L-third:H   -1.0340225 -4.103090 2.0350455 0.9208238
second:L-first:L   0.5867562 -2.482312 3.6558242 0.9932590
third:L-first:L   -0.6739975 -3.743065 2.3950705 0.9872725
third:L-second:L  -1.2607537 -4.329822 1.8083143 0.8341226
```



```
$`treatment:time:hl`
                                   diff        lwr      upr     p adj
CT:first:H-BSA:first:H    -1.448922e+00  -8.018431 5.120587 0.9999962
NH:first:H-BSA:first:H     1.038760e+00  -5.530750 7.608269 1.0000000
BSA:second:H-BSA:first:H   1.738919e+00  -4.830590 8.308429 0.9999489
CT:second:H-BSA:first:H   -6.239531e-01  -7.193462 5.945556 1.0000000
NH:second:H-BSA:first:H   -2.936424e-01  -6.863152 6.275867 1.0000000
BSA:third:H-BSA:first:H   -2.264056e+00  -8.833565 4.305453 0.9984510
CT:third:H-BSA:first:H    -1.938660e+00  -8.508169 4.630850 0.9997791
NH:third:H-BSA:first:H     2.204166e-01  -6.349093 6.789926 1.0000000
BSA:first:L-BSA:first:H   -2.094991e+00  -8.664500 4.474519 0.9994017
CT:first:L-BSA:first:H    -2.377621e+00  -8.947131 4.191888 0.9972497
NH:first:L-BSA:first:H    -5.897625e-01  -7.159272 5.979747 1.0000000
BSA:second:L-BSA:first:H   1.570682e-01  -6.412441 6.726578 1.0000000
CT:second:L-BSA:first:H   -6.679495e-01  -7.237459 5.901560 1.0000000
NH:second:L-BSA:first:H   -2.791224e+00  -9.360734 3.778285 0.9845835
BSA:third:L-BSA:first:H   -2.141604e+00  -8.711113 4.427905 0.9992126
CT:third:L-BSA:first:H    -2.151538e+00  -8.721048 4.417971 0.9991662
NH:third:L-BSA:first:H    -2.791224e+00  -9.360734 3.778285 0.9845835
NH:first:H-CT:first:H      2.487682e+00  -4.081828 9.057191 0.9954120
BSA:second:H-CT:first:H    3.187841e+00  -3.381668 9.757350 0.9477457
CT:second:H-CT:first:H     8.249688e-01  -5.744541 7.394478 1.0000000
NH:second:H-CT:first:H     1.155279e+00  -5.414230 7.724789 0.9999999
BSA:third:H-CT:first:H    -8.151341e-01  -7.384643 5.754375 1.0000000
CT:third:H-CT:first:H     -4.897378e-01  -7.059247 6.079772 1.0000000
NH:third:H-CT:first:H      1.669339e+00  -4.900171 8.238848 0.9999711
BSA:first:L-CT:first:H    -6.460686e-01  -7.215578 5.923441 1.0000000
CT:first:L-CT:first:H     -9.286992e-01  -7.498209 5.640810 1.0000000
NH:first:L-CT:first:H      8.591594e-01  -5.710350 7.428669 1.0000000
BSA:second:L-CT:first:H    1.605990e+00  -4.963519 8.175499 0.9999832
CT:second:L-CT:first:H     7.809724e-01  -5.788537 7.350482 1.0000000
NH:second:L-CT:first:H    -1.342302e+00  -7.911812 5.227207 0.9999988
BSA:third:L-CT:first:H    -6.926820e-01  -7.262191 5.876827 1.0000000
CT:third:L-CT:first:H     -7.026165e-01  -7.272126 5.866893 1.0000000
NH:third:L-CT:first:H     -1.342302e+00  -7.911812 5.227207 0.9999988
BSA:second:H-NH:first:H    7.001593e-01  -5.869350 7.269669 1.0000000
CT:second:H-NH:first:H    -1.662713e+00  -8.232222 4.906796 0.9999726
NH:second:H-NH:first:H    -1.332402e+00  -7.901912 5.237107 0.9999989
BSA:third:H-NH:first:H    -3.302816e+00  -9.872325 3.266694 0.9301320
CT:third:H-NH:first:H     -2.977420e+00  -9.546929 3.592090 0.9713849
NH:third:H-NH:first:H     -8.183432e-01  -7.387853 5.751166 1.0000000
BSA:first:L-NH:first:H    -3.133750e+00  -9.703260 3.435759 0.9548326
CT:first:L-NH:first:H     -3.416381e+00  -9.985890 3.153128 0.9091391
NH:first:L-NH:first:H     -1.628522e+00  -8.198032 4.940987 0.9999796
BSA:second:L-NH:first:H   -8.816916e-01  -7.451201 5.687818 1.0000000
CT:second:L-NH:first:H    -1.706709e+00  -8.276219 4.862800 0.9999606
NH:second:L-NH:first:H    -3.829984e+00 -10.399494 2.739525 0.8017165
BSA:third:L-NH:first:H    -3.180364e+00  -9.749873 3.389146 0.9487697
CT:third:L-NH:first:H     -3.190298e+00  -9.759808 3.379211 0.9474060
NH:third:L-NH:first:H     -3.829984e+00 -10.399494 2.739525 0.8017165
CT:second:H-BSA:second:H  -2.362872e+00  -8.932382 4.206637 0.9974403
NH:second:H-BSA:second:H  -2.032562e+00  -8.602071 4.536948 0.9995923
BSA:third:H-BSA:second:H  -4.002975e+00 -10.572484 2.566534 0.7438299
CT:third:H-BSA:second:H   -3.677579e+00 -10.247088 2.891931 0.8468010
NH:third:H-BSA:second:H   -1.518503e+00  -8.088012 5.051007 0.9999925
BSA:first:L-BSA:second:H  -3.833910e+00 -10.403419 2.735600 0.8004774
CT:first:L-BSA:second:H   -4.116540e+00 -10.686050 2.452969 0.7026800
NH:first:L-BSA:second:H   -2.328682e+00  -8.898191 4.240828 0.9978396
BSA:second:L-BSA:second:H -1.581851e+00  -8.151360 4.987658 0.9999865
CT:second:L-BSA:second:H  -2.406869e+00  -8.976378 4.162641 0.9968360
NH:second:L-BSA:second:H  -4.530143e+00 -11.099653 2.039366 0.5416050
BSA:third:L-BSA:second:H  -3.880523e+00 -10.450032 2.688986 0.7854856
CT:third:L-BSA:second:H   -3.890458e+00 -10.459967 2.679052 0.7822255
NH:third:L-BSA:second:H   -4.530143e+00 -11.099653 2.039366 0.5416050
```



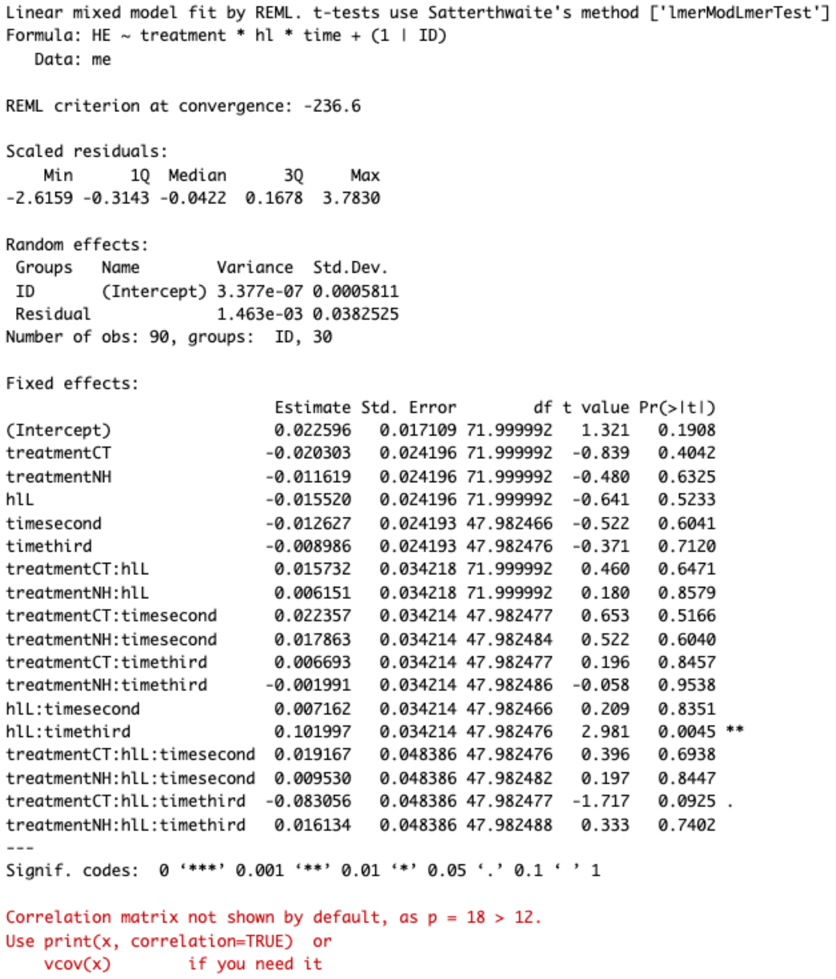
```
NH:second:H-CT:second:H    3.303106e-01  -6.239199 6.899820 1.0000000
BSA:third:H-CT:second:H   -1.640103e+00  -8.209612 4.929406 0.9999774
CT:third:H-CT:second:H    -1.314707e+00  -7.884216 5.254803 0.9999991
NH:third:H-CT:second:H     8.443697e-01  -5.725140 7.413879 1.0000000
BSA:first:L-CT:second:H   -1.471037e+00  -8.040547 5.098472 0.9999953
CT:first:L-CT:second:H    -1.753668e+00  -8.323177 4.815841 0.9999426
NH:first:L-CT:second:H     3.419055e-02  -6.535319 6.603700 1.0000000
BSA:second:L-CT:second:H   7.810213e-01  -5.788488 7.350531 1.0000000
CT:second:L-CT:second:H   -4.399640e-02  -6.613506 6.525513 1.0000000
NH:second:L-CT:second:H   -2.167271e+00  -8.736781 4.402238 0.9990879
BSA:third:L-CT:second:H   -1.517651e+00  -8.087160 5.051859 0.9999926
CT:third:L-CT:second:H    -1.527585e+00  -8.097095 5.041924 0.9999918
NH:third:L-CT:second:H    -2.167271e+00  -8.736781 4.402238 0.9990879
BSA:third:H-NH:second:H   -1.970414e+00  -8.539923 4.599096 0.9997268
CT:third:H-NH:second:H    -1.645017e+00  -8.214527 4.924492 0.9999765
NH:third:H-NH:second:H     5.140591e-01  -6.055450 7.083568 1.0000000
BSA:first:L-NH:second:H   -1.801348e+00  -8.370857 4.768161 0.9999172
CT:first:L-NH:second:H    -2.083979e+00  -8.653488 4.485531 0.9994401
NH:first:L-NH:second:H    -2.961201e-01  -6.865629 6.273389 1.0000000
BSA:second:L-NH:second:H   4.507107e-01  -6.118799 7.020220 1.0000000
CT:second:L-NH:second:H   -3.743070e-01  -6.943816 6.195202 1.0000000
NH:second:L-NH:second:H   -2.497582e+00  -9.067091 4.071928 0.9952056
BSA:third:L-NH:second:H   -1.847961e+00  -8.417471 4.721548 0.9998831
CT:third:L-NH:second:H    -1.857896e+00  -8.427405 4.711613 0.9998743
NH:third:L-NH:second:H    -2.497582e+00  -9.067091 4.071928 0.9952056
CT:third:H-BSA:third:H     3.253963e-01  -6.244113 6.894906 1.0000000
NH:third:H-BSA:third:H     2.484473e+00  -4.085037 9.053982 0.9954773
BSA:first:L-BSA:third:H    1.690655e-01  -6.400444 6.738575 1.0000000
CT:first:L-BSA:third:H    -1.135652e-01  -6.683075 6.455944 1.0000000
NH:first:L-BSA:third:H     1.674293e+00  -4.895216 8.243803 0.9999698
BSA:second:L-BSA:third:H   2.421124e+00  -4.148385 8.990634 0.9966161
CT:second:L-BSA:third:H    1.596107e+00  -4.973403 8.165616 0.9999847
NH:second:L-BSA:third:H   -5.271683e-01  -7.096678 6.042341 1.0000000
BSA:third:L-BSA:third:H    1.224521e-01  -6.447057 6.691961 1.0000000
CT:third:L-BSA:third:H     1.125176e-01  -6.456992 6.682027 1.0000000
NH:third:L-BSA:third:H    -5.271683e-01  -7.096678 6.042341 1.0000000
NH:third:H-CT:third:H      2.159076e+00  -4.410433 8.728586 0.9991294
BSA:first:L-CT:third:H    -1.563308e-01  -6.725840 6.413179 1.0000000
CT:first:L-CT:third:H     -4.389615e-01  -7.008471 6.130548 1.0000000
NH:first:L-CT:third:H      1.348897e+00  -5.220612 7.918407 0.9999987
BSA:second:L-CT:third:H    2.095728e+00  -4.473781 8.665237 0.9993990
CT:second:L-CT:third:H     1.270710e+00  -5.298799 7.840220 0.9999995
NH:second:L-CT:third:H    -8.525646e-01  -7.422074 5.716945 1.0000000
BSA:third:L-CT:third:H    -2.029442e-01  -6.772454 6.366565 1.0000000
CT:third:L-CT:third:H     -2.128787e-01  -6.782388 6.356631 1.0000000
NH:third:L-CT:third:H     -8.525646e-01  -7.422074 5.716945 1.0000000
BSA:first:L-NH:third:H    -2.315407e+00  -8.884917 4.254102 0.9979796
CT:first:L-NH:third:H     -2.598038e+00  -9.167547 3.971472 0.9926400
NH:first:L-NH:third:H     -8.101792e-01  -7.379689 5.759330 1.0000000
BSA:second:L-NH:third:H   -6.334841e-02  -6.632858 6.506161 1.0000000
CT:second:L-NH:third:H    -8.883661e-01  -7.457875 5.681143 1.0000000
NH:second:L-NH:third:H    -3.011641e+00  -9.581150 3.557868 0.9682305
BSA:third:L-NH:third:H    -2.362021e+00  -8.931530 4.207489 0.9974509
CT:third:L-NH:third:H     -2.371955e+00  -8.941464 4.197554 0.9973243
NH:third:L-NH:third:H     -3.011641e+00  -9.581150 3.557868 0.9682305
CT:first:L-BSA:first:L    -2.826306e-01  -6.852140 6.286879 1.0000000
NH:first:L-BSA:first:L     1.505228e+00  -5.064281 8.074737 0.9999934
BSA:second:L-BSA:first:L   2.252059e+00  -4.317451 8.821568 0.9985464
CT:second:L-BSA:first:L    1.427041e+00  -5.142468 7.996550 0.9999970
NH:second:L-BSA:first:L   -6.962338e-01  -7.265743 5.873276 1.0000000
BSA:third:L-BSA:first:L   -4.661340e-02  -6.616123 6.522896 1.0000000
CT:third:L-BSA:first:L    -5.654786e-02  -6.626057 6.512962 1.0000000
```



```
NH:third:L-BSA:first:L    -6.962338e-01  -7.265743 5.873276 1.0000000
NH:first:L-CT:first:L      1.787859e+00  -4.781651 8.357368 0.9999252
BSA:second:L-CT:first:L    2.534689e+00  -4.034820 9.104199 0.9943620
CT:second:L-CT:first:L     1.709672e+00  -4.859838 8.279181 0.9999596
NH:second:L-CT:first:L    -4.136031e-01  -6.983113 6.155906 1.0000000
BSA:third:L-CT:first:L     2.360172e-01  -6.333492 6.805527 1.0000000
CT:third:L-CT:first:L      2.260828e-01  -6.343427 6.795592 1.0000000
NH:third:L-CT:first:L     -4.136031e-01  -6.983113 6.155906 1.0000000
BSA:second:L-NH:first:L    7.468307e-01  -5.822679 7.316340 1.0000000
CT:second:L-NH:first:L    -7.818694e-02  -6.647696 6.491322 1.0000000
NH:second:L-NH:first:L    -2.201462e+00  -8.770971 4.368048 0.9988953
BSA:third:L-NH:first:L    -1.551841e+00  -8.121351 5.017668 0.9999897
CT:third:L-NH:first:L     -1.561776e+00  -8.131285 5.007734 0.9999888
NH:third:L-NH:first:L     -2.201462e+00  -8.770971 4.368048 0.9988953
CT:second:L-BSA:second:L  -8.250177e-01  -7.394527 5.744492 1.0000000
NH:second:L-BSA:second:L  -2.948292e+00  -9.517802 3.621217 0.9738778
BSA:third:L-BSA:second:L  -2.298672e+00  -8.868181 4.270837 0.9981451
CT:third:L-BSA:second:L   -2.308607e+00  -8.878116 4.260903 0.9980483
NH:third:L-BSA:second:L   -2.948292e+00  -9.517802 3.621217 0.9738778
NH:second:L-CT:second:L   -2.123275e+00  -8.692784 4.446235 0.9992924
BSA:third:L-CT:second:L   -1.473654e+00  -8.043164 5.095855 0.9999952
CT:third:L-CT:second:L    -1.483589e+00  -8.053098 5.085920 0.9999947
NH:third:L-CT:second:L    -2.123275e+00  -8.692784 4.446235 0.9992924
BSA:third:L-NH:second:L    6.496204e-01  -5.919889 7.219130 1.0000000
CT:third:L-NH:second:L     6.396859e-01  -5.929823 7.209195 1.0000000
NH:third:L-NH:second:L    -5.967449e-15  -6.569509 6.569509 1.0000000
CT:third:L-BSA:third:L    -9.934463e-03  -6.579444 6.559575 1.0000000
NH:third:L-BSA:third:L    -6.496204e-01  -7.219130 5.919889 1.0000000
NH:third:L-CT:third:L     -6.396859e-01  -7.209195 5.929823 1.0000000
```

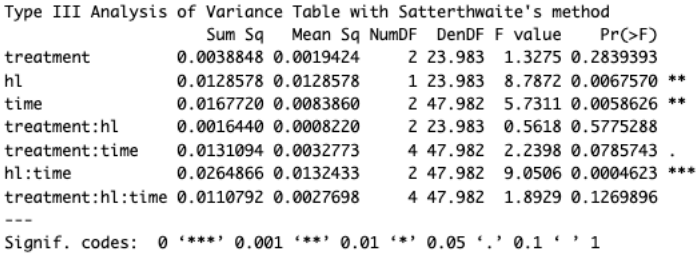
Figure 26. Tukey test of MnP activity in Mycena, interactions time, medium and concentration.

Mycena peptidase



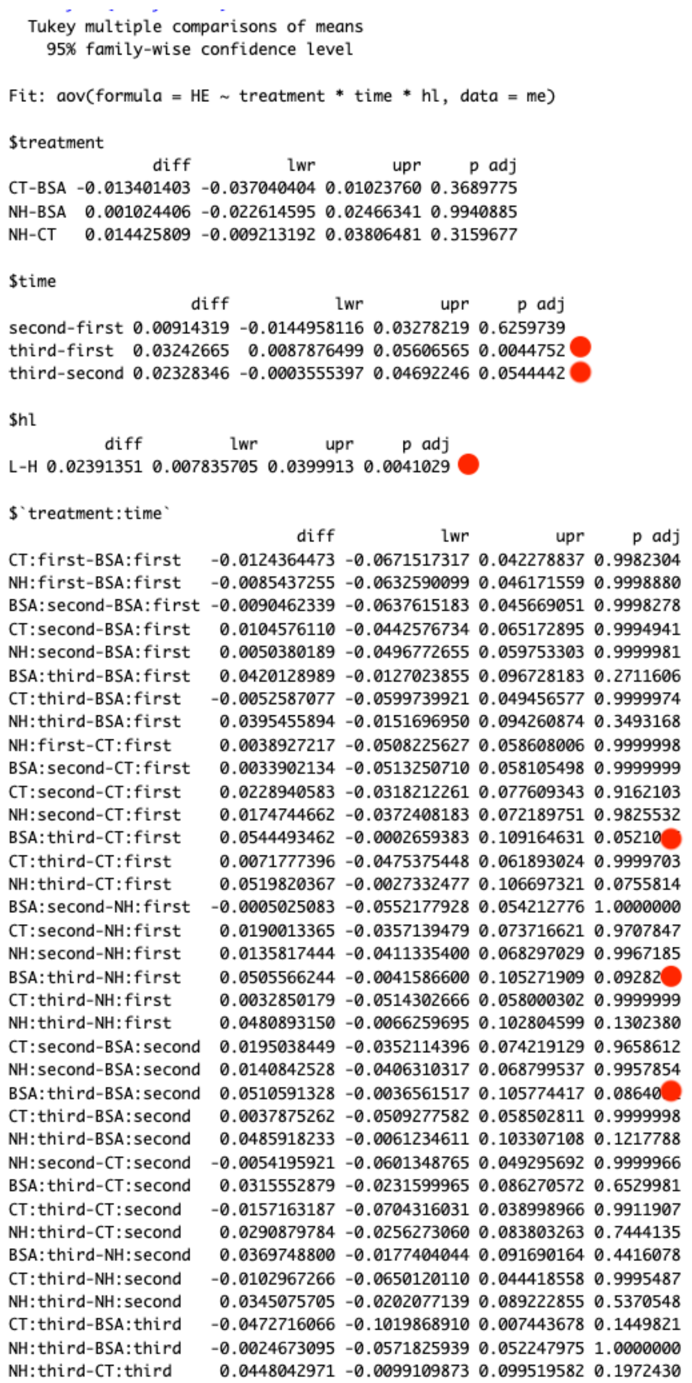
```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: HE ~ treatment * hl * time + (1 | ID)
   Data: me

REML criterion at convergence: -236.6

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-2.6159 -0.3143 -0.0422  0.1678  3.7830 

Random effects:
 Groups   Name        Variance  Std.Dev.
 ID       (Intercept) 3.377e-07 0.0005811
 Residual             1.463e-03 0.0382525
Number of obs: 90, groups:  ID, 30

Fixed effects:
                            Estimate Std. Error        df t value Pr(>|t|)   
(Intercept)                 0.022596   0.017109 71.999992   1.321   0.1908   
treatmentCT                -0.020303   0.024196 71.999992  -0.839   0.4042   
treatmentNH                -0.011619   0.024196 71.999992  -0.480   0.6325   
hlL                        -0.015520   0.024196 71.999992  -0.641   0.5233   
timesecond                 -0.012627   0.024193 47.982466  -0.522   0.6041   
timethird                  -0.008986   0.024193 47.982476  -0.371   0.7120   
treatmentCT:hlL             0.015732   0.034218 71.999992   0.460   0.6471   
treatmentNH:hlL             0.006151   0.034218 71.999992   0.180   0.8579   
treatmentCT:timesecond      0.022357   0.034214 47.982477   0.653   0.5166   
treatmentNH:timesecond      0.017863   0.034214 47.982484   0.522   0.6040   
treatmentCT:timethird       0.006693   0.034214 47.982477   0.196   0.8457   
treatmentNH:timethird      -0.001991   0.034214 47.982486  -0.058   0.9538   
hlL:timesecond              0.007162   0.034214 47.982466   0.209   0.8351   
hlL:timethird               0.101997   0.034214 47.982476   2.981   0.0045 **
treatmentCT:hlL:timesecond  0.019167   0.048386 47.982476   0.396   0.6938   
treatmentNH:hlL:timesecond  0.009530   0.048386 47.982482   0.197   0.8447   
treatmentCT:hlL:timethird  -0.083056   0.048386 47.982477  -1.717   0.0925 . 
treatmentNH:hlL:timethird   0.016134   0.048386 47.982488   0.333   0.7402   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation matrix not shown by default, as p = 18 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it
```

Figure 27. Linear mixed model of peptidase activity in Mycena, interactions time, medium and concentration.

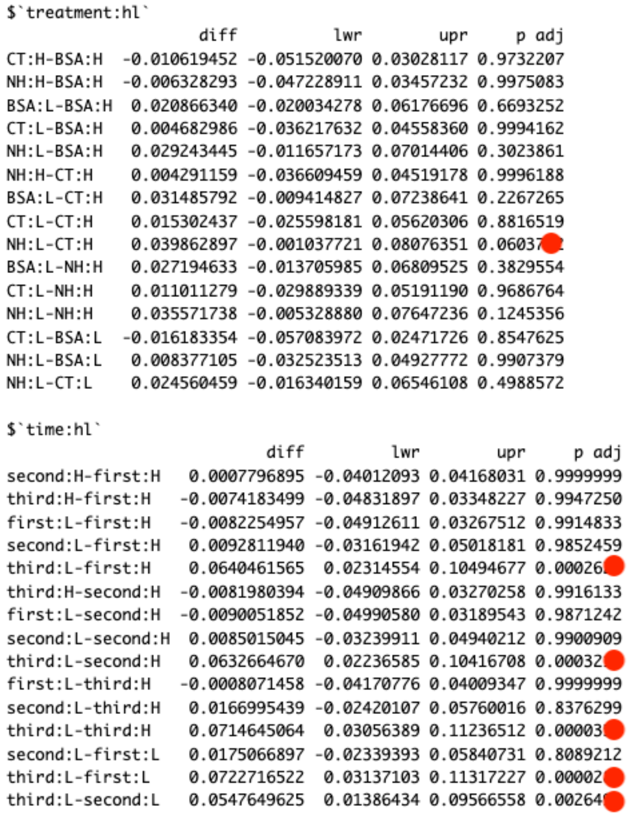


```
Type III Analysis of Variance Table with Satterthwaite's method
                      Sum Sq   Mean Sq NumDF  DenDF F value    Pr(>F)    
treatment          0.0038848 0.0019424     2 23.983  1.3275 0.2839393    
hl                 0.0128578 0.0128578     1 23.983  8.7872 0.0067570 ** 
time               0.0167720 0.0083860     2 47.982  5.7311 0.0058626 ** 
treatment:hl       0.0016440 0.0008220     2 23.983  0.5618 0.5775288    
treatment:time     0.0131094 0.0032773     4 47.982  2.2398 0.0785743 .  
hl:time            0.0264866 0.0132433     2 47.982  9.0506 0.0004623 ***
treatment:hl:time  0.0110792 0.0027698     4 47.982  1.8929 0.1269896    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

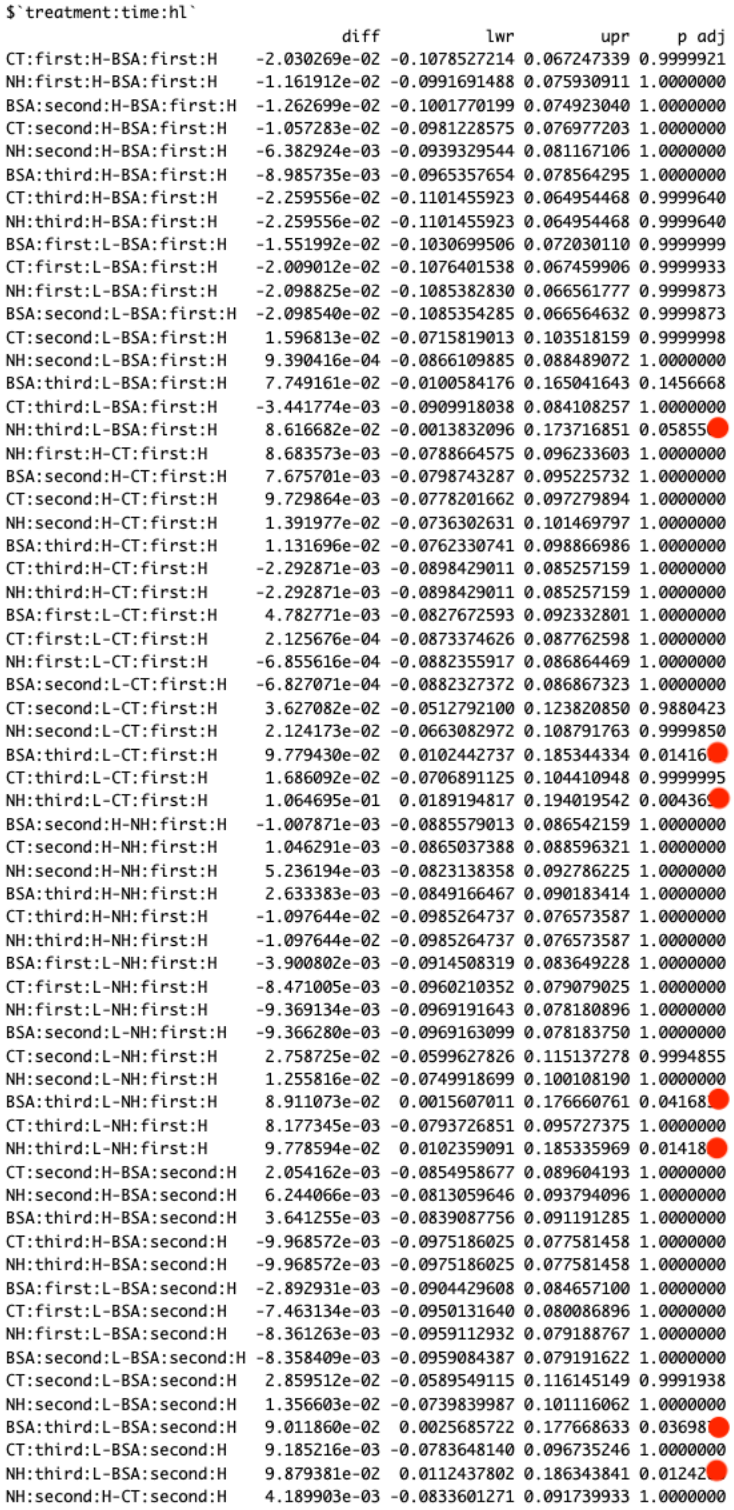
Figure 28. ANOVA of peptidase activity in Mycena, interactions time, medium and concentration.



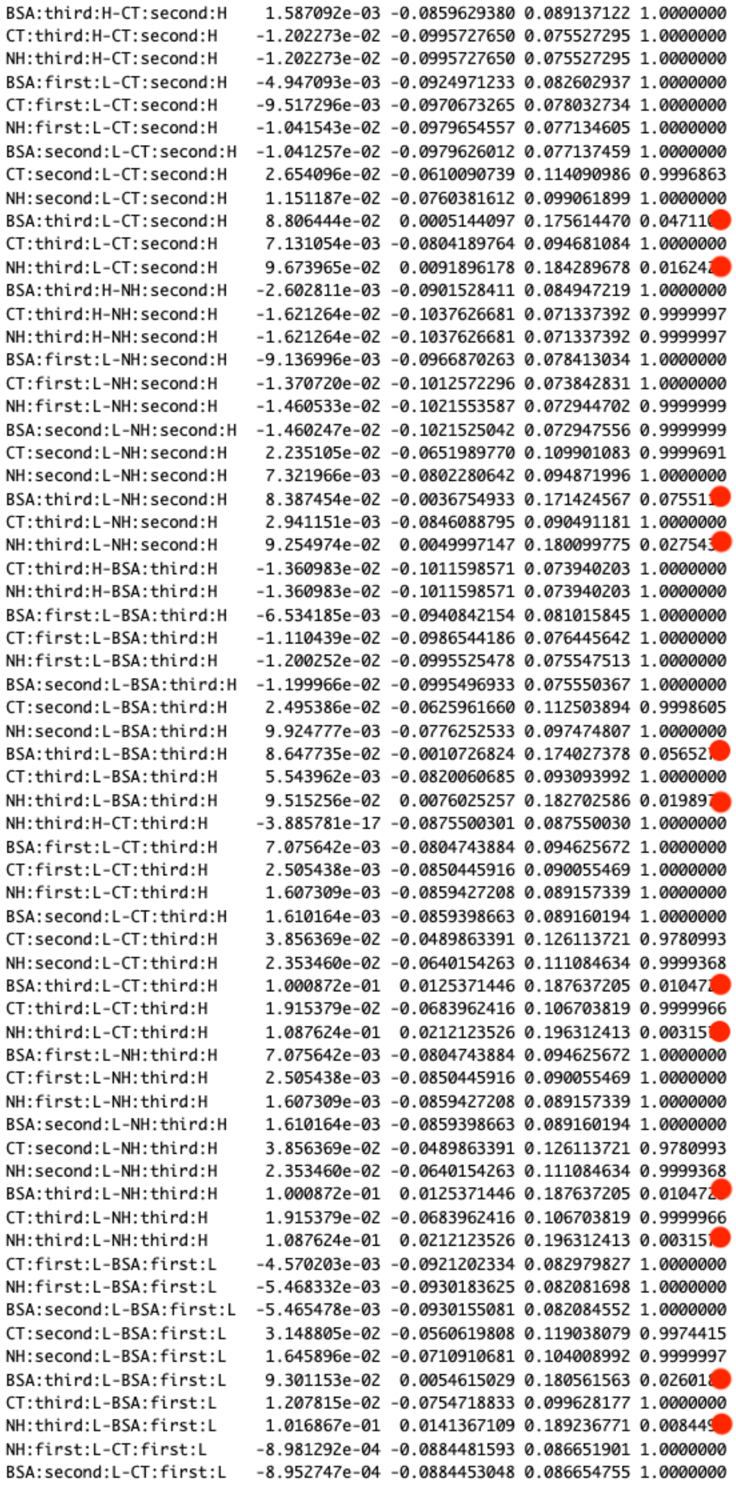
```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = HE ~ treatment * time * hl, data = me)

$treatment
               diff          lwr        upr     p adj
CT-BSA -0.013401403 -0.037040404 0.01023760 0.3689775
NH-BSA  0.001024406 -0.022614595 0.02466341 0.9940885
NH-CT   0.014425809 -0.009213192 0.03806481 0.3159677

$time
                   diff           lwr        upr     p adj
second-first 0.00914319 -0.0144958116 0.03278219 0.6259739
third-first  0.03242665  0.0087876499 0.05606565 0.0044752 ●
third-second 0.02328346 -0.0003555397 0.04692246 0.0544442 ●

$hl
          diff         lwr       upr     p adj
L-H 0.02391351 0.007835705 0.0399913 0.0041029 ●

$`treatment:time`
                              diff           lwr         upr     p adj
CT:first-BSA:first    -0.0124364473 -0.0671517317 0.042278837 0.9982304
NH:first-BSA:first    -0.0085437255 -0.0632590099 0.046171559 0.9998880
BSA:second-BSA:first -0.0090462339 -0.0637615183 0.045669051 0.9998278
CT:second-BSA:first    0.0104576110 -0.0442576734 0.065172895 0.9994941
NH:second-BSA:first    0.0050380189 -0.0496772655 0.059753303 0.9999981
BSA:third-BSA:first    0.0420128989 -0.0127023855 0.096728183 0.2711606
CT:third-BSA:first    -0.0052587077 -0.0599739921 0.049456577 0.9999974
NH:third-BSA:first     0.0395455894 -0.0151696950 0.094260874 0.3493168
NH:first-CT:first      0.0038927217 -0.0508225627 0.058608006 0.9999998
BSA:second-CT:first    0.0033902134 -0.0513250710 0.058105498 0.9999999
CT:second-CT:first     0.0228940583 -0.0318212261 0.077609343 0.9162103
NH:second-CT:first     0.0174744662 -0.0372408183 0.072189751 0.9825532
BSA:third-CT:first     0.0544493462 -0.0002659383 0.109164631 0.05210●
CT:third-CT:first      0.0071777396 -0.0475375448 0.061893024 0.9999703
NH:third-CT:first      0.0519820367 -0.0027332477 0.106697321 0.0755814
BSA:second-NH:first   -0.0005025083 -0.0552177928 0.054212776 1.0000000
CT:second-NH:first     0.0190013365 -0.0357139479 0.073716621 0.9707847
NH:second-NH:first     0.0135817444 -0.0411335400 0.068297029 0.9967185
BSA:third-NH:first     0.0505566244 -0.0041586600 0.105271909 0.09282●
CT:third-NH:first      0.0032850179 -0.0514302666 0.058000302 0.9999999
NH:third-NH:first      0.0480893150 -0.0066259695 0.102804599 0.1302380
CT:second-BSA:second   0.0195038449 -0.0352114396 0.074219129 0.9658612
NH:second-BSA:second   0.0140842528 -0.0406310317 0.068799537 0.9957854
BSA:third-BSA:second   0.0510591328 -0.0036561517 0.105774417 0.08640●
CT:third-BSA:second    0.0037875262 -0.0509277582 0.058502811 0.9999998
NH:third-BSA:second    0.0485918233 -0.0061234611 0.103307108 0.1217788
NH:second-CT:second   -0.0054195921 -0.0601348765 0.049295692 0.9999966
BSA:third-CT:second    0.0315552879 -0.0231599965 0.086270572 0.6529981
CT:third-CT:second    -0.0157163187 -0.0704316031 0.038998966 0.9911907
NH:third-CT:second     0.0290879784 -0.0256273060 0.083803263 0.7444135
BSA:third-NH:second    0.0369748800 -0.0177404044 0.091690164 0.4416078
CT:third-NH:second    -0.0102967266 -0.0650120110 0.044418558 0.9995487
NH:third-NH:second     0.0345075705 -0.0202077139 0.089222855 0.5370548
CT:third-BSA:third    -0.0472716066 -0.1019868910 0.007443678 0.1449821
NH:third-BSA:third    -0.0024673095 -0.0571825939 0.052247975 1.0000000
NH:third-CT:third      0.0448042971 -0.0099109873 0.099519582 0.1972430
```



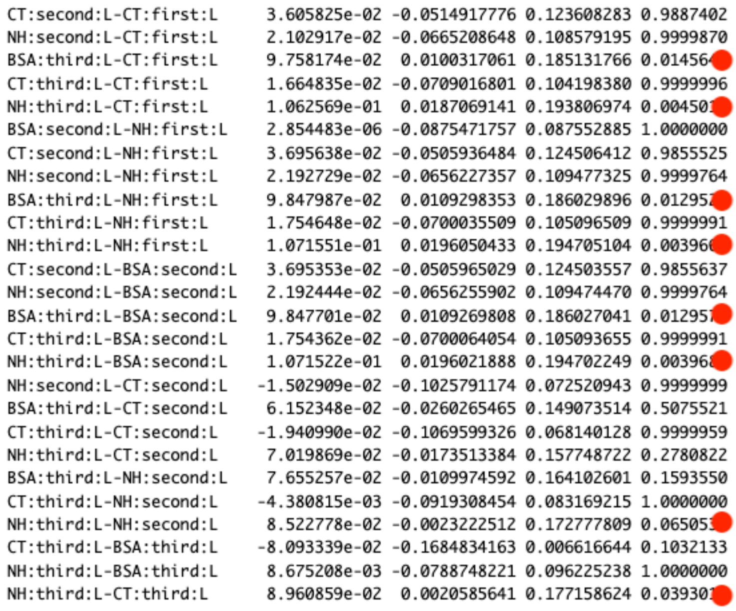
```
$`treatment:hl`
                    diff          lwr        upr     p adj
CT:H-BSA:H  -0.010619452 -0.051520070 0.03028117 0.9732207
NH:H-BSA:H  -0.006328293 -0.047228911 0.03457232 0.9975083
BSA:L-BSA:H  0.020866340 -0.020034278 0.06176696 0.6693252
CT:L-BSA:H   0.004682986 -0.036217632 0.04558360 0.9994162
NH:L-BSA:H   0.029243445 -0.011657173 0.07014406 0.3023861
NH:H-CT:H    0.004291159 -0.036609459 0.04519178 0.9996188
BSA:L-CT:H   0.031485792 -0.009414827 0.07238641 0.2267265
CT:L-CT:H    0.015302437 -0.025598181 0.05620306 0.8816519
NH:L-CT:H    0.039862897 -0.001037721 0.08076351 0.06037●
BSA:L-NH:H   0.027194633 -0.013705985 0.06809525 0.3829554
CT:L-NH:H    0.011011279 -0.029889339 0.05191190 0.9686764
NH:L-NH:H    0.035571738 -0.005328880 0.07647236 0.1245356
CT:L-BSA:L  -0.016183354 -0.057083972 0.02471726 0.8547625
NH:L-BSA:L   0.008377105 -0.032523513 0.04927772 0.9907379
NH:L-CT:L    0.024560459 -0.016340159 0.06546108 0.4988572

$`time:hl`
                           diff         lwr        upr     p adj
second:H-first:H   0.0007796895 -0.04012093 0.04168031 0.9999999
third:H-first:H   -0.0074183499 -0.04831897 0.03348227 0.9947250
first:L-first:H   -0.0082254957 -0.04912611 0.03267512 0.9914833
second:L-first:H   0.0092811940 -0.03161942 0.05018181 0.9852459
third:L-first:H    0.0640461565  0.02314554 0.10494677 0.00026●
third:H-second:H  -0.0081980394 -0.04909866 0.03270258 0.9916133
first:L-second:H  -0.0090051852 -0.04990580 0.03189543 0.9871242
second:L-second:H  0.0085015045 -0.03239911 0.04940212 0.9900909
third:L-second:H   0.0632664670  0.02236585 0.10416708 0.00032●
first:L-third:H   -0.0008071458 -0.04170776 0.04009347 0.9999999
second:L-third:H   0.0166995439 -0.02420107 0.05760016 0.8376299
third:L-third:H    0.0714645064  0.03056389 0.11236512 0.00003●
second:L-first:L   0.0175066897 -0.02339393 0.05840731 0.8089212
third:L-first:L    0.0722716522  0.03137103 0.11317227 0.00002●
third:L-second:L   0.0547649625  0.01386434 0.09566558 0.00264●
```



```
$`treatment:time:hl`
                                diff          lwr         upr     p adj
CT:first:H-BSA:first:H    -2.030269e-02 -0.1078527214 0.067247339 0.9999921
NH:first:H-BSA:first:H    -1.161912e-02 -0.0991691488 0.075930911 1.0000000
BSA:second:H-BSA:first:H  -1.262699e-02 -0.1001770199 0.074923040 1.0000000
CT:second:H-BSA:first:H   -1.057283e-02 -0.0981228575 0.076977203 1.0000000
NH:second:H-BSA:first:H   -6.382924e-03 -0.0939329544 0.081167106 1.0000000
BSA:third:H-BSA:first:H   -8.985735e-03 -0.0965357654 0.078564295 1.0000000
CT:third:H-BSA:first:H    -2.259556e-02 -0.1101455923 0.064954468 0.9999640
NH:third:H-BSA:first:H    -2.259556e-02 -0.1101455923 0.064954468 0.9999640
BSA:first:L-BSA:first:H   -1.551992e-02 -0.1030699506 0.072030110 0.9999999
CT:first:L-BSA:first:H    -2.009012e-02 -0.1076401538 0.067459906 0.9999933
NH:first:L-BSA:first:H    -2.098825e-02 -0.1085382830 0.066561777 0.9999873
BSA:second:L-BSA:first:H  -2.098540e-02 -0.1085354285 0.066564632 0.9999873
CT:second:L-BSA:first:H    1.596813e-02 -0.0715819013 0.103518159 0.9999998
NH:second:L-BSA:first:H    9.390416e-04 -0.0866109885 0.088489072 1.0000000
BSA:third:L-BSA:first:H    7.749161e-02 -0.0100584176 0.165041643 0.1456668
CT:third:L-BSA:first:H    -3.441774e-03 -0.0909918038 0.084108257 1.0000000
NH:third:L-BSA:first:H     8.616682e-02 -0.0013832096 0.173716851 0.05855●
NH:first:H-CT:first:H      8.683573e-03 -0.0788664575 0.096233603 1.0000000
BSA:second:H-CT:first:H    7.675701e-03 -0.0798743287 0.095225732 1.0000000
CT:second:H-CT:first:H     9.729864e-03 -0.0778201662 0.097279894 1.0000000
NH:second:H-CT:first:H     1.391977e-02 -0.0736302631 0.101469797 1.0000000
BSA:third:H-CT:first:H     1.131696e-02 -0.0762330741 0.098866986 1.0000000
CT:third:H-CT:first:H     -2.292871e-03 -0.0898429011 0.085257159 1.0000000
NH:third:H-CT:first:H     -2.292871e-03 -0.0898429011 0.085257159 1.0000000
BSA:first:L-CT:first:H     4.782771e-03 -0.0827672593 0.092332801 1.0000000
CT:first:L-CT:first:H      2.125676e-04 -0.0873374626 0.087762598 1.0000000
NH:first:L-CT:first:H     -6.855616e-04 -0.0882355917 0.086864469 1.0000000
BSA:second:L-CT:first:H   -6.827071e-04 -0.0882327372 0.086867323 1.0000000
CT:second:L-CT:first:H     3.627082e-02 -0.0512792100 0.123820850 0.9880423
NH:second:L-CT:first:H     2.124173e-02 -0.0663082972 0.108791763 0.9999850
BSA:third:L-CT:first:H     9.779430e-02  0.0102442737 0.185344334 0.01416●
CT:third:L-CT:first:H      1.686092e-02 -0.0706891125 0.104410948 0.9999995
NH:third:L-CT:first:H      1.064695e-01  0.0189194817 0.194019542 0.00436●
BSA:second:H-NH:first:H   -1.007871e-03 -0.0885579013 0.086542159 1.0000000
CT:second:H-NH:first:H     1.046291e-03 -0.0865037388 0.088596321 1.0000000
NH:second:H-NH:first:H     5.236194e-03 -0.0823138358 0.092786225 1.0000000
BSA:third:H-NH:first:H     2.633383e-03 -0.0849166467 0.090183414 1.0000000
CT:third:H-NH:first:H     -1.097644e-02 -0.0985264737 0.076573587 1.0000000
NH:third:H-NH:first:H     -1.097644e-02 -0.0985264737 0.076573587 1.0000000
BSA:first:L-NH:first:H    -3.900802e-03 -0.0914508319 0.083649228 1.0000000
CT:first:L-NH:first:H     -8.471005e-03 -0.0960210352 0.079079025 1.0000000
NH:first:L-NH:first:H     -9.369134e-03 -0.0969191643 0.078180896 1.0000000
BSA:second:L-NH:first:H   -9.366280e-03 -0.0969163099 0.078183750 1.0000000
CT:second:L-NH:first:H     2.758725e-02 -0.0599627826 0.115137278 0.9994855
NH:second:L-NH:first:H     1.255816e-02 -0.0749918699 0.100108190 1.0000000
BSA:third:L-NH:first:H     8.911073e-02  0.0015607011 0.176660761 0.04168●
CT:third:L-NH:first:H      8.177345e-03 -0.0793726851 0.095727375 1.0000000
NH:third:L-NH:first:H      9.778594e-02  0.0102359091 0.185335969 0.01418●
CT:second:H-BSA:second:H   2.054162e-03 -0.0854958677 0.089604193 1.0000000
NH:second:H-BSA:second:H   6.244066e-03 -0.0813059646 0.093794096 1.0000000
BSA:third:H-BSA:second:H   3.641255e-03 -0.0839087756 0.091191285 1.0000000
CT:third:H-BSA:second:H   -9.968572e-03 -0.0975186025 0.077581458 1.0000000
NH:third:H-BSA:second:H   -9.968572e-03 -0.0975186025 0.077581458 1.0000000
BSA:first:L-BSA:second:H  -2.892931e-03 -0.0904429608 0.084657100 1.0000000
CT:first:L-BSA:second:H   -7.463134e-03 -0.0950131640 0.080086896 1.0000000
NH:first:L-BSA:second:H   -8.361263e-03 -0.0959112932 0.079188767 1.0000000
BSA:second:L-BSA:second:H -8.358409e-03 -0.0959084387 0.079191622 1.0000000
CT:second:L-BSA:second:H   2.859512e-02 -0.0589549115 0.116145149 0.9991938
NH:second:L-BSA:second:H   1.356603e-02 -0.0739839987 0.101116062 1.0000000
BSA:third:L-BSA:second:H   9.011860e-02  0.0025685722 0.177668633 0.03698●
CT:third:L-BSA:second:H    9.185216e-03 -0.0783648140 0.096735246 1.0000000
NH:third:L-BSA:second:H    9.879381e-02  0.0112437802 0.186343841 0.01242●
NH:second:H-CT:second:H    4.189903e-03 -0.0833601271 0.091739933 1.0000000
```



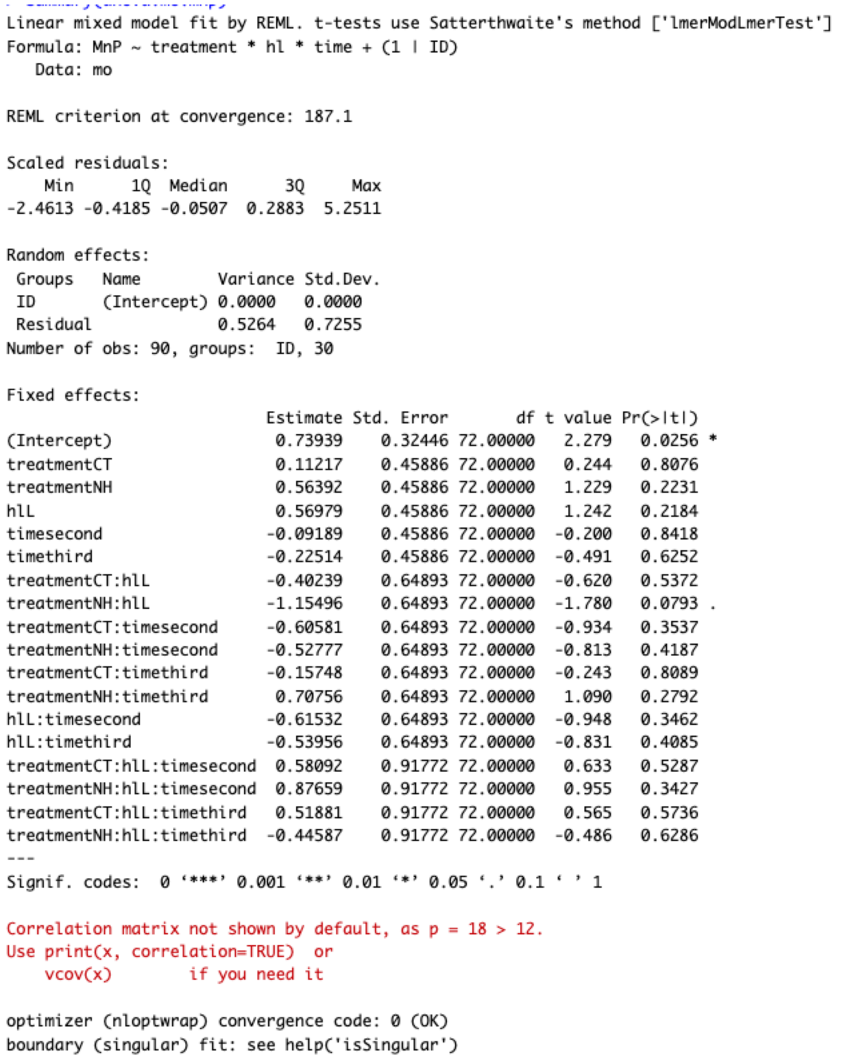
```
BSA:third:H-CT:second:H    1.587092e-03 -0.0859629380 0.089137122 1.0000000
CT:third:H-CT:second:H    -1.202273e-02 -0.0995727650 0.075527295 1.0000000
NH:third:H-CT:second:H    -1.202273e-02 -0.0995727650 0.075527295 1.0000000
BSA:first:L-CT:second:H   -4.947093e-03 -0.0924971233 0.082602937 1.0000000
CT:first:L-CT:second:H    -9.517296e-03 -0.0970673265 0.078032734 1.0000000
NH:first:L-CT:second:H    -1.041543e-02 -0.0979654557 0.077134605 1.0000000
BSA:second:L-CT:second:H  -1.041257e-02 -0.0979626012 0.077137459 1.0000000
CT:second:L-CT:second:H    2.654096e-02 -0.0610090739 0.114090986 0.9996863
NH:second:L-CT:second:H    1.151187e-02 -0.0760381612 0.099061899 1.0000000
BSA:third:L-CT:second:H    8.806444e-02  0.0005144097 0.175614470 0.04711●
CT:third:L-CT:second:H     7.131054e-03 -0.0804189764 0.094681084 1.0000000
NH:third:L-CT:second:H     9.673965e-02  0.0091896178 0.184289678 0.01624●
BSA:third:H-NH:second:H   -2.602811e-03 -0.0901528411 0.084947219 1.0000000
CT:third:H-NH:second:H    -1.621264e-02 -0.1037626681 0.071337392 0.9999997
NH:third:H-NH:second:H    -1.621264e-02 -0.1037626681 0.071337392 0.9999997
BSA:first:L-NH:second:H   -9.136996e-03 -0.0966870263 0.078413034 1.0000000
CT:first:L-NH:second:H    -1.370720e-02 -0.1012572296 0.073842831 1.0000000
NH:first:L-NH:second:H    -1.460533e-02 -0.1021553587 0.072944702 0.9999999
BSA:second:L-NH:second:H  -1.460247e-02 -0.1021525042 0.072947556 0.9999999
CT:second:L-NH:second:H    2.235105e-02 -0.0651989770 0.109901083 0.9999691
NH:second:L-NH:second:H    7.321966e-03 -0.0802280642 0.094871996 1.0000000
BSA:third:L-NH:second:H    8.387454e-02 -0.0036754933 0.171424567 0.07551●
CT:third:L-NH:second:H     2.941151e-03 -0.0846088795 0.090491181 1.0000000
NH:third:L-NH:second:H     9.254974e-02  0.0049997147 0.180099775 0.02754●
CT:third:H-BSA:third:H    -1.360983e-02 -0.1011598571 0.073940203 1.0000000
NH:third:H-BSA:third:H    -1.360983e-02 -0.1011598571 0.073940203 1.0000000
BSA:first:L-BSA:third:H   -6.534185e-03 -0.0940842154 0.081015845 1.0000000
CT:first:L-BSA:third:H    -1.110439e-02 -0.0986544186 0.076445642 1.0000000
NH:first:L-BSA:third:H    -1.200252e-02 -0.0995525478 0.075547513 1.0000000
BSA:second:L-BSA:third:H  -1.199966e-02 -0.0995496933 0.075550367 1.0000000
CT:second:L-BSA:third:H    2.495386e-02 -0.0625961660 0.112503894 0.9998605
NH:second:L-BSA:third:H    9.924777e-03 -0.0776252533 0.097474807 1.0000000
BSA:third:L-BSA:third:H    8.647735e-02 -0.0010726824 0.174027378 0.05652●
CT:third:L-BSA:third:H     5.543962e-03 -0.0820060685 0.093093992 1.0000000
NH:third:L-BSA:third:H     9.515256e-02  0.0076025257 0.182702586 0.01989●
NH:third:H-CT:third:H     -3.885781e-17 -0.0875500301 0.087550030 1.0000000
BSA:first:L-CT:third:H     7.075642e-03 -0.0804743884 0.094625672 1.0000000
CT:first:L-CT:third:H      2.505438e-03 -0.0850445916 0.090055469 1.0000000
NH:first:L-CT:third:H      1.607309e-03 -0.0859427208 0.089157339 1.0000000
BSA:second:L-CT:third:H    1.610164e-03 -0.0859398663 0.089160194 1.0000000
CT:second:L-CT:third:H     3.856369e-02 -0.0489863391 0.126113721 0.9780993
NH:second:L-CT:third:H     2.353460e-02 -0.0640154263 0.111084634 0.9999368
BSA:third:L-CT:third:H     1.000872e-01  0.0125371446 0.187637205 0.01047●
CT:third:L-CT:third:H      1.915379e-02 -0.0683962416 0.106703819 0.9999966
NH:third:L-CT:third:H      1.087624e-01  0.0212123526 0.196312413 0.00315●
BSA:first:L-NH:third:H     7.075642e-03 -0.0804743884 0.094625672 1.0000000
CT:first:L-NH:third:H      2.505438e-03 -0.0850445916 0.090055469 1.0000000
NH:first:L-NH:third:H      1.607309e-03 -0.0859427208 0.089157339 1.0000000
BSA:second:L-NH:third:H    1.610164e-03 -0.0859398663 0.089160194 1.0000000
CT:second:L-NH:third:H     3.856369e-02 -0.0489863391 0.126113721 0.9780993
NH:second:L-NH:third:H     2.353460e-02 -0.0640154263 0.111084634 0.9999368
BSA:third:L-NH:third:H     1.000872e-01  0.0125371446 0.187637205 0.01047●
CT:third:L-NH:third:H      1.915379e-02 -0.0683962416 0.106703819 0.9999966
NH:third:L-NH:third:H      1.087624e-01  0.0212123526 0.196312413 0.00315●
CT:first:L-BSA:first:L    -4.570203e-03 -0.0921202334 0.082979827 1.0000000
NH:first:L-BSA:first:L    -5.468332e-03 -0.0930183625 0.082081698 1.0000000
BSA:second:L-BSA:first:L  -5.465478e-03 -0.0930155081 0.082084552 1.0000000
CT:second:L-BSA:first:L    3.148805e-02 -0.0560619808 0.119038079 0.9974415
NH:second:L-BSA:first:L    1.645896e-02 -0.0710910681 0.104008992 0.9999997
BSA:third:L-BSA:first:L    9.301153e-02  0.0054615029 0.180561563 0.02601●
CT:third:L-BSA:first:L     1.207815e-02 -0.0754718833 0.099628177 1.0000000
NH:third:L-BSA:first:L     1.016867e-01  0.0141367109 0.189236771 0.00844●
NH:first:L-CT:first:L     -8.981292e-04 -0.0884481593 0.086651901 1.0000000
BSA:second:L-CT:first:L   -8.952747e-04 -0.0884453048 0.086654755 1.0000000
```



```
CT:second:L-CT:first:L      3.605825e-02 -0.0514917776 0.123608283 0.9887402
NH:second:L-CT:first:L      2.102917e-02 -0.0665208648 0.108579195 0.9999870
BSA:third:L-CT:first:L      9.758174e-02  0.0100317061 0.185131766 0.014564
CT:third:L-CT:first:L       1.664835e-02 -0.0709016801 0.104198380 0.9999996
NH:third:L-CT:first:L       1.062569e-01  0.0187069141 0.193806974 0.004501
BSA:second:L-NH:first:L     2.854483e-06 -0.0875471757 0.087552885 1.0000000
CT:second:L-NH:first:L      3.695638e-02 -0.0505936484 0.124506412 0.9855525
NH:second:L-NH:first:L      2.192729e-02 -0.0656227357 0.109477325 0.9999764
BSA:third:L-NH:first:L      9.847987e-02  0.0109298353 0.186029896 0.012952
CT:third:L-NH:first:L       1.754648e-02 -0.0700035509 0.105096509 0.9999991
NH:third:L-NH:first:L       1.071551e-01  0.0196050433 0.194705104 0.003960
CT:second:L-BSA:second:L    3.695353e-02 -0.0505965029 0.124503557 0.9855637
NH:second:L-BSA:second:L    2.192444e-02 -0.0656255902 0.109474470 0.9999764
BSA:third:L-BSA:second:L    9.847701e-02  0.0109269808 0.186027041 0.012957
CT:third:L-BSA:second:L     1.754362e-02 -0.0700064054 0.105093655 0.9999991
NH:third:L-BSA:second:L     1.071522e-01  0.0196021888 0.194702249 0.003968
NH:second:L-CT:second:L    -1.502909e-02 -0.1025791174 0.072520943 0.9999999
BSA:third:L-CT:second:L     6.152348e-02 -0.0260265465 0.149073514 0.5075521
CT:third:L-CT:second:L     -1.940990e-02 -0.1069599326 0.068140128 0.9999959
NH:third:L-CT:second:L      7.019869e-02 -0.0173513384 0.157748722 0.2780822
BSA:third:L-NH:second:L     7.655257e-02 -0.0109974592 0.164102601 0.1593550
CT:third:L-NH:second:L     -4.380815e-03 -0.0919308454 0.083169215 1.0000000
NH:third:L-NH:second:L      8.522778e-02 -0.0023222512 0.172777809 0.065053
CT:third:L-BSA:third:L     -8.093339e-02 -0.1684834163 0.006616644 0.1032133
NH:third:L-BSA:third:L      8.675208e-03 -0.0788748221 0.096225238 1.0000000
NH:third:L-CT:third:L       8.960859e-02  0.0020585641 0.177158624 0.039301
```

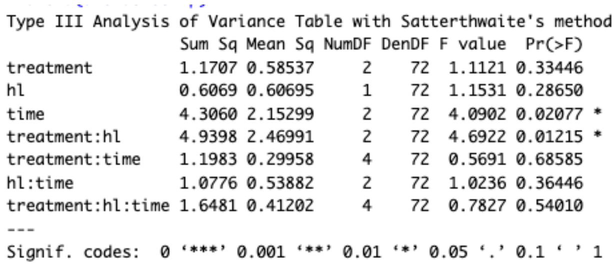
Figure 29. Tukey test of peptidase activity in Mycena, interactions time, medium and concentration.

Marasmius MnP



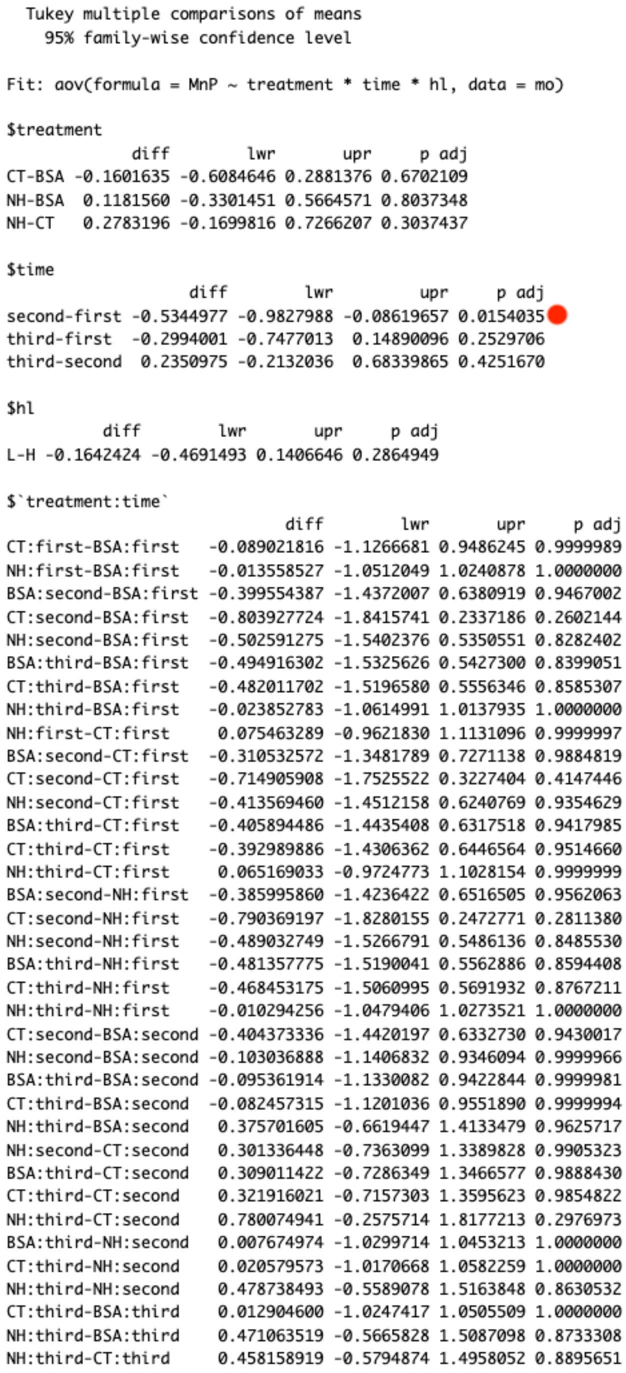
```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: MnP ~ treatment * hl * time + (1 | ID)
   Data: mo

REML criterion at convergence: 187.1

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-2.4613 -0.4185 -0.0507  0.2883  5.2511 

Random effects:
 Groups   Name        Variance Std.Dev.
 ID       (Intercept) 0.0000   0.0000  
 Residual             0.5264   0.7255  
Number of obs: 90, groups:  ID, 30

Fixed effects:
                           Estimate Std. Error       df t value Pr(>|t|)  
(Intercept)                 0.73939    0.32446 72.00000   2.279   0.0256 *
treatmentCT                 0.11217    0.45886 72.00000   0.244   0.8076  
treatmentNH                 0.56392    0.45886 72.00000   1.229   0.2231  
hlL                         0.56979    0.45886 72.00000   1.242   0.2184  
timesecond                 -0.09189    0.45886 72.00000  -0.200   0.8418  
timethird                  -0.22514    0.45886 72.00000  -0.491   0.6252  
treatmentCT:hlL            -0.40239    0.64893 72.00000  -0.620   0.5372  
treatmentNH:hlL            -1.15496    0.64893 72.00000  -1.780   0.0793 .
treatmentCT:timesecond     -0.60581    0.64893 72.00000  -0.934   0.3537  
treatmentNH:timesecond     -0.52777    0.64893 72.00000  -0.813   0.4187  
treatmentCT:timethird      -0.15748    0.64893 72.00000  -0.243   0.8089  
treatmentNH:timethird       0.70756    0.64893 72.00000   1.090   0.2792  
hlL:timesecond             -0.61532    0.64893 72.00000  -0.948   0.3462  
hlL:timethird              -0.53956    0.64893 72.00000  -0.831   0.4085  
treatmentCT:hlL:timesecond  0.58092    0.91772 72.00000   0.633   0.5287  
treatmentNH:hlL:timesecond  0.87659    0.91772 72.00000   0.955   0.3427  
treatmentCT:hlL:timethird   0.51881    0.91772 72.00000   0.565   0.5736  
treatmentNH:hlL:timethird  -0.44587    0.91772 72.00000  -0.486   0.6286  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation matrix not shown by default, as p = 18 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it

optimizer (nloptwrap) convergence code: 0 (OK)
boundary (singular) fit: see help('isSingular')
```

Figure 30. Linear mixed model of MnP activity in Marasmius, interactions time, medium and concentration.

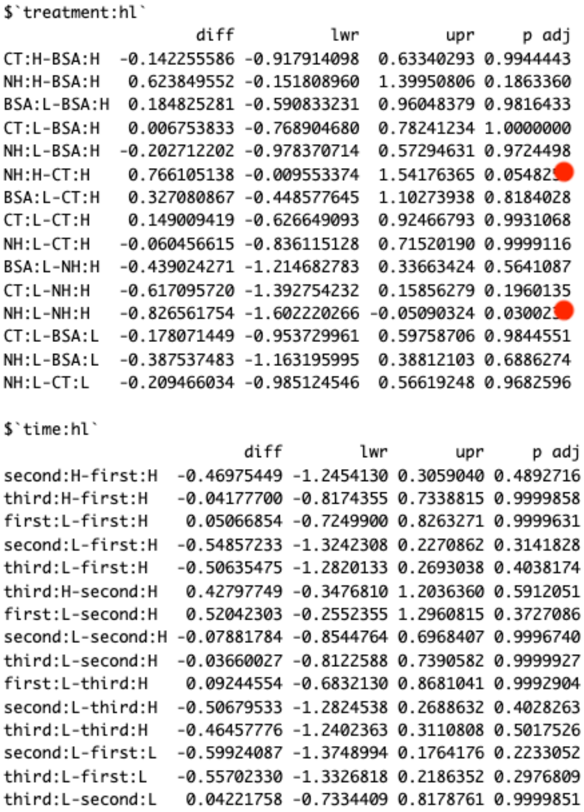


```
Type III Analysis of Variance Table with Satterthwaite's method
                  Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)  
treatment         1.1707 0.58537     2    72  1.1121 0.33446  
hl                0.6069 0.60695     1    72  1.1531 0.28650  
time              4.3060 2.15299     2    72  4.0902 0.02077 *
treatment:hl      4.9398 2.46991     2    72  4.6922 0.01215 *
treatment:time    1.1983 0.29958     4    72  0.5691 0.68585  
hl:time           1.0776 0.53882     2    72  1.0236 0.36446  
treatment:hl:time 1.6481 0.41202     4    72  0.7827 0.54010  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

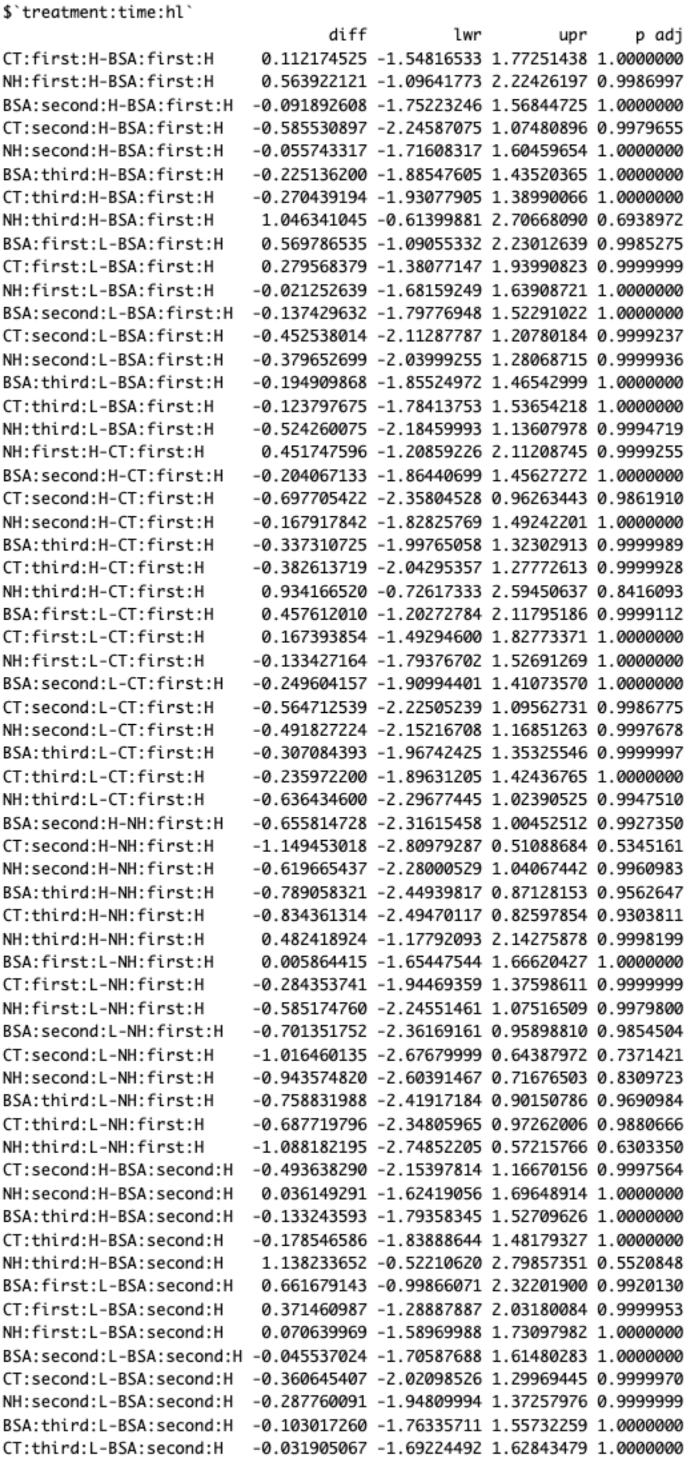
Figure 31. ANOVA of MnP activity in Marasmius, interactions time, medium and concentration.



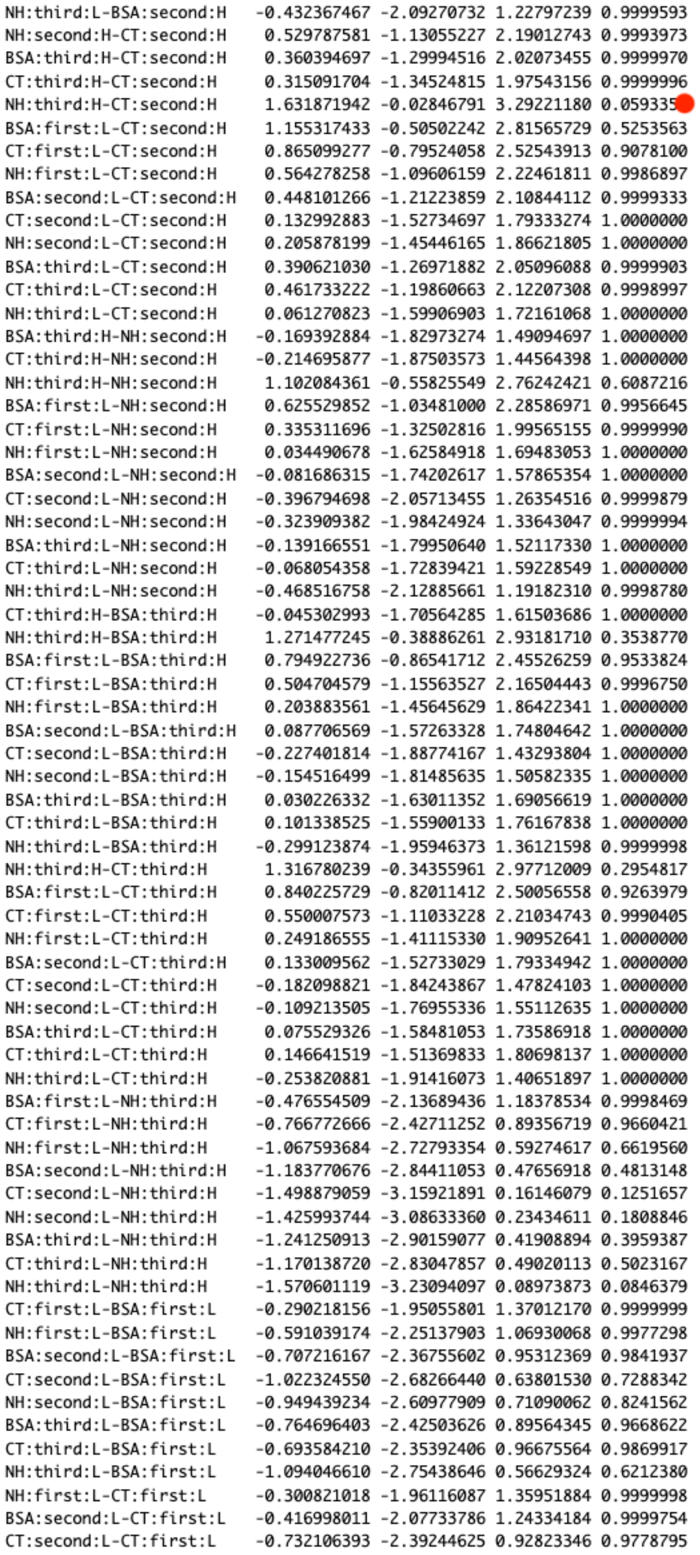
```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = MnP ~ treatment * time * hl, data = mo)

$treatment
             diff        lwr       upr     p adj
CT-BSA -0.1601635 -0.6084646 0.2881376 0.6702109
NH-BSA  0.1181560 -0.3301451 0.5664571 0.8037348
NH-CT   0.2783196 -0.1699816 0.7266207 0.3037437

$time
                   diff        lwr         upr     p adj
second-first -0.5344977 -0.9827988 -0.08619657 0.0154035●
third-first  -0.2994001 -0.7477013  0.14890096 0.2529706
third-second  0.2350975 -0.2132036  0.68339865 0.4251670

$hl
          diff        lwr       upr     p adj
L-H -0.1642424 -0.4691493 0.1406646 0.2864949

$`treatment:time`
                             diff        lwr       upr     p adj
CT:first-BSA:first   -0.089021816 -1.1266681 0.9486245 0.9999989
NH:first-BSA:first   -0.013558527 -1.0512049 1.0240878 1.0000000
BSA:second-BSA:first -0.399554387 -1.4372007 0.6380919 0.9467002
CT:second-BSA:first  -0.803927724 -1.8415741 0.2337186 0.2602144
NH:second-BSA:first  -0.502591275 -1.5402376 0.5350551 0.8282402
BSA:third-BSA:first  -0.494916302 -1.5325626 0.5427300 0.8399051
CT:third-BSA:first   -0.482011702 -1.5196580 0.5556346 0.8585307
NH:third-BSA:first   -0.023852783 -1.0614991 1.0137935 1.0000000
NH:first-CT:first     0.075463289 -0.9621830 1.1131096 0.9999997
BSA:second-CT:first  -0.310532572 -1.3481789 0.7271138 0.9884819
CT:second-CT:first   -0.714905908 -1.7525522 0.3227404 0.4147446
NH:second-CT:first   -0.413569460 -1.4512158 0.6240769 0.9354629
BSA:third-CT:first   -0.405894486 -1.4435408 0.6317518 0.9417985
CT:third-CT:first    -0.392989886 -1.4306362 0.6446564 0.9514660
NH:third-CT:first     0.065169033 -0.9724773 1.1028154 0.9999999
BSA:second-NH:first  -0.385995860 -1.4236422 0.6516505 0.9562063
CT:second-NH:first   -0.790369197 -1.8280155 0.2472771 0.2811380
NH:second-NH:first   -0.489032749 -1.5266791 0.5486136 0.8485530
BSA:third-NH:first   -0.481357775 -1.5190041 0.5562886 0.8594408
CT:third-NH:first    -0.468453175 -1.5060995 0.5691932 0.8767211
NH:third-NH:first    -0.010294256 -1.0479406 1.0273521 1.0000000
CT:second-BSA:second -0.404373336 -1.4420197 0.6332730 0.9430017
NH:second-BSA:second -0.103036888 -1.1406832 0.9346094 0.9999966
BSA:third-BSA:second -0.095361914 -1.1330082 0.9422844 0.9999981
CT:third-BSA:second  -0.082457315 -1.1201036 0.9551890 0.9999994
NH:third-BSA:second   0.375701605 -0.6619447 1.4133479 0.9625717
NH:second-CT:second   0.301336448 -0.7363099 1.3389828 0.9905323
BSA:third-CT:second   0.309011422 -0.7286349 1.3466577 0.9888430
CT:third-CT:second    0.321916021 -0.7157303 1.3595623 0.9854822
NH:third-CT:second    0.780074941 -0.2575714 1.8177213 0.2976973
BSA:third-NH:second   0.007674974 -1.0299714 1.0453213 1.0000000
CT:third-NH:second    0.020579573 -1.0170668 1.0582259 1.0000000
NH:third-NH:second    0.478738493 -0.5589078 1.5163848 0.8630532
CT:third-BSA:third    0.012904600 -1.0247417 1.0505509 1.0000000
NH:third-BSA:third    0.471063519 -0.5665828 1.5087098 0.8733308
NH:third-CT:third     0.458158919 -0.5794874 1.4958052 0.8895651
```



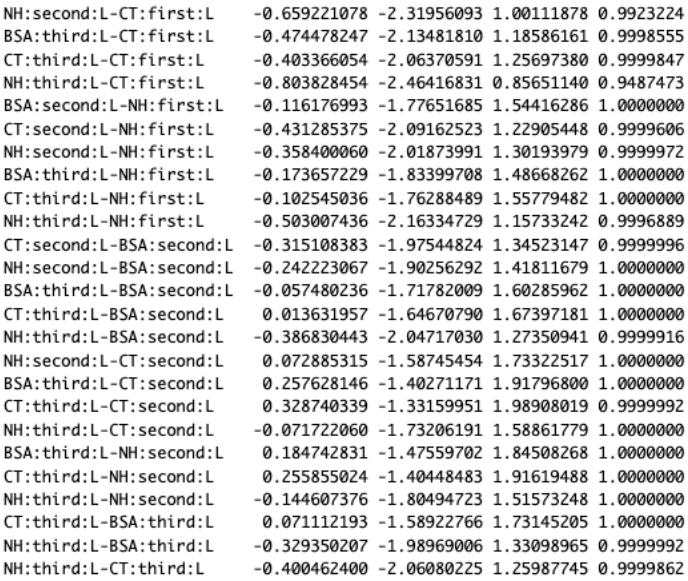
```
$`treatment:hl`
                   diff          lwr         upr     p adj
CT:H-BSA:H  -0.142255586 -0.917914098  0.63340293 0.9944443
NH:H-BSA:H   0.623849552 -0.151808960  1.39950806 0.1863360
BSA:L-BSA:H  0.184825281 -0.590833231  0.96048379 0.9816433
CT:L-BSA:H   0.006753833 -0.768904680  0.78241234 1.0000000
NH:L-BSA:H  -0.202712202 -0.978370714  0.57294631 0.9724498
NH:H-CT:H    0.766105138 -0.009553374  1.54176365 0.054825
BSA:L-CT:H   0.327080867 -0.448577645  1.10273938 0.8184028
CT:L-CT:H    0.149009419 -0.626649093  0.92466793 0.9931068
NH:L-CT:H   -0.060456615 -0.836115128  0.71520190 0.9999116
BSA:L-NH:H  -0.439024271 -1.214682783  0.33663424 0.5641087
CT:L-NH:H   -0.617095720 -1.392754232  0.15856279 0.1960135
NH:L-NH:H   -0.826561754 -1.602220266 -0.05090324 0.030022
CT:L-BSA:L  -0.178071449 -0.953729961  0.59758706 0.9844551
NH:L-BSA:L  -0.387537483 -1.163195995  0.38812103 0.6886274
NH:L-CT:L   -0.209466034 -0.985124546  0.56619248 0.9682596

$`time:hl`
                        diff        lwr       upr     p adj
second:H-first:H  -0.46975449 -1.2454130 0.3059040 0.4892716
third:H-first:H   -0.04177700 -0.8174355 0.7338815 0.9999858
first:L-first:H    0.05066854 -0.7249900 0.8263271 0.9999631
second:L-first:H  -0.54857233 -1.3242308 0.2270862 0.3141828
third:L-first:H   -0.50635475 -1.2820133 0.2693038 0.4038174
third:H-second:H   0.42797749 -0.3476810 1.2036360 0.5912051
first:L-second:H   0.52042303 -0.2552355 1.2960815 0.3727086
second:L-second:H -0.07881784 -0.8544764 0.6968407 0.9996740
third:L-second:H  -0.03660027 -0.8122588 0.7390582 0.9999927
first:L-third:H    0.09244554 -0.6832130 0.8681041 0.9992904
second:L-third:H  -0.50679533 -1.2824538 0.2688632 0.4028263
third:L-third:H   -0.46457776 -1.2402363 0.3110808 0.5017526
second:L-first:L  -0.59924087 -1.3748994 0.1764176 0.2233052
third:L-first:L   -0.55702330 -1.3326818 0.2186352 0.2976809
third:L-second:L   0.04221758 -0.7334409 0.8178761 0.9999851
```



```
$`treatment:time:hl`
                                  diff         lwr        upr     p adj
CT:first:H-BSA:first:H     0.112174525 -1.54816533 1.77251438 1.0000000
NH:first:H-BSA:first:H     0.563922121 -1.09641773 2.22426197 0.9986997
BSA:second:H-BSA:first:H  -0.091892608 -1.75223246 1.56844725 1.0000000
CT:second:H-BSA:first:H   -0.585530897 -2.24587075 1.07480896 0.9979655
NH:second:H-BSA:first:H   -0.055743317 -1.71608317 1.60459654 1.0000000
BSA:third:H-BSA:first:H   -0.225136200 -1.88547605 1.43520365 1.0000000
CT:third:H-BSA:first:H    -0.270439194 -1.93077905 1.38990066 1.0000000
NH:third:H-BSA:first:H     1.046341045 -0.61399881 2.70668090 0.6938972
BSA:first:L-BSA:first:H    0.569786535 -1.09055332 2.23012639 0.9985275
CT:first:L-BSA:first:H     0.279568379 -1.38077147 1.93990823 0.9999999
NH:first:L-BSA:first:H    -0.021252639 -1.68159249 1.63908721 1.0000000
BSA:second:L-BSA:first:H  -0.137429632 -1.79776948 1.52291022 1.0000000
CT:second:L-BSA:first:H   -0.452538014 -2.11287787 1.20780184 0.9999237
NH:second:L-BSA:first:H   -0.379652699 -2.03999255 1.28068715 0.9999936
BSA:third:L-BSA:first:H   -0.194909868 -1.85524972 1.46542999 1.0000000
CT:third:L-BSA:first:H    -0.123797675 -1.78413753 1.53654218 1.0000000
NH:third:L-BSA:first:H    -0.524260075 -2.18459993 1.13607978 0.9994719
NH:first:H-CT:first:H      0.451747596 -1.20859226 2.11208745 0.9999255
BSA:second:H-CT:first:H   -0.204067133 -1.86440699 1.45627272 1.0000000
CT:second:H-CT:first:H    -0.697705422 -2.35804528 0.96263443 0.9861910
NH:second:H-CT:first:H    -0.167917842 -1.82825769 1.49242201 1.0000000
BSA:third:H-CT:first:H    -0.337310725 -1.99765058 1.32302913 0.9999989
CT:third:H-CT:first:H     -0.382613719 -2.04295357 1.27772613 0.9999928
NH:third:H-CT:first:H      0.934166520 -0.72617333 2.59450637 0.8416093
BSA:first:L-CT:first:H     0.457612010 -1.20272784 2.11795186 0.9999112
CT:first:L-CT:first:H      0.167393854 -1.49294600 1.82773371 1.0000000
NH:first:L-CT:first:H     -0.133427164 -1.79376702 1.52691269 1.0000000
BSA:second:L-CT:first:H   -0.249604157 -1.90994401 1.41073570 1.0000000
CT:second:L-CT:first:H    -0.564712539 -2.22505239 1.09562731 0.9986775
NH:second:L-CT:first:H    -0.491827224 -2.15216708 1.16851263 0.9997678
BSA:third:L-CT:first:H    -0.307084393 -1.96742425 1.35325546 0.9999997
CT:third:L-CT:first:H     -0.235972200 -1.89631205 1.42436765 1.0000000
NH:third:L-CT:first:H     -0.636434600 -2.29677445 1.02390525 0.9947510
BSA:second:H-NH:first:H   -0.655814728 -2.31615458 1.00452512 0.9927350
CT:second:H-NH:first:H    -1.149453018 -2.80979287 0.51088684 0.5345161
NH:second:H-NH:first:H    -0.619665437 -2.28000529 1.04067442 0.9960983
BSA:third:H-NH:first:H    -0.789058321 -2.44939817 0.87128153 0.9562647
CT:third:H-NH:first:H     -0.834361314 -2.49470117 0.82597854 0.9303811
NH:third:H-NH:first:H      0.482418924 -1.17792093 2.14275878 0.9998199
BSA:first:L-NH:first:H     0.005864415 -1.65447544 1.66620427 1.0000000
CT:first:L-NH:first:H     -0.284353741 -1.94469359 1.37598611 0.9999999
NH:first:L-NH:first:H     -0.585174760 -2.24551461 1.07516509 0.9979800
BSA:second:L-NH:first:H   -0.701351752 -2.36169161 0.95898810 0.9854504
CT:second:L-NH:first:H    -1.016460135 -2.67679999 0.64387972 0.7371421
NH:second:L-NH:first:H    -0.943574820 -2.60391467 0.71676503 0.8309723
BSA:third:L-NH:first:H    -0.758831988 -2.41917184 0.90150786 0.9690984
CT:third:L-NH:first:H     -0.687719796 -2.34805965 0.97262006 0.9880666
NH:third:L-NH:first:H     -1.088182195 -2.74852205 0.57215766 0.6303350
CT:second:H-BSA:second:H  -0.493638290 -2.15397814 1.16670156 0.9997564
NH:second:H-BSA:second:H   0.036149291 -1.62419056 1.69648914 1.0000000
BSA:third:H-BSA:second:H  -0.133243593 -1.79358345 1.52709626 1.0000000
CT:third:H-BSA:second:H   -0.178546586 -1.83888644 1.48179327 1.0000000
NH:third:H-BSA:second:H    1.138233652 -0.52210620 2.79857351 0.5520848
BSA:first:L-BSA:second:H   0.661679143 -0.99866071 2.32201900 0.9920130
CT:first:L-BSA:second:H    0.371460987 -1.28887887 2.03180084 0.9999953
NH:first:L-BSA:second:H    0.070639969 -1.58969988 1.73097982 1.0000000
BSA:second:L-BSA:second:H -0.045537024 -1.70587688 1.61480283 1.0000000
CT:second:L-BSA:second:H  -0.360645407 -2.02098526 1.29969445 0.9999970
NH:second:L-BSA:second:H  -0.287760091 -1.94809994 1.37257976 0.9999999
BSA:third:L-BSA:second:H  -0.103017260 -1.76335711 1.55732259 1.0000000
CT:third:L-BSA:second:H   -0.031905067 -1.69224492 1.62843479 1.0000000
```



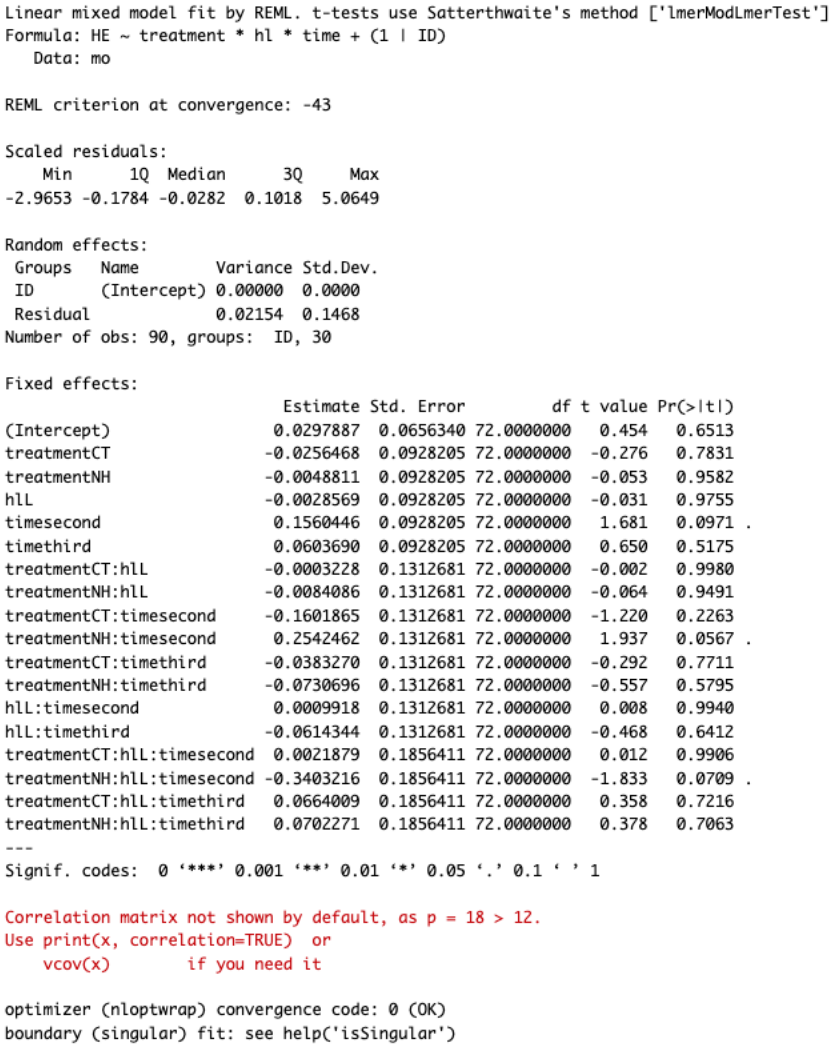
```
NH:third:L-BSA:second:H  -0.432367467 -2.09270732 1.22797239 0.9999593
NH:second:H-CT:second:H   0.529787581 -1.13055227 2.19012743 0.9993973
BSA:third:H-CT:second:H   0.360394697 -1.29994516 2.02073455 0.9999970
CT:third:H-CT:second:H    0.315091704 -1.34524815 1.97543156 0.9999996
NH:third:H-CT:second:H    1.631871942 -0.02846791 3.29221180 0.059335
BSA:first:L-CT:second:H   1.155317433 -0.50502242 2.81565729 0.5253563
CT:first:L-CT:second:H    0.865099277 -0.79524058 2.52543913 0.9078100
NH:first:L-CT:second:H    0.564278258 -1.09606159 2.22461811 0.9986897
BSA:second:L-CT:second:H  0.448101266 -1.21223859 2.10844112 0.9999333
CT:second:L-CT:second:H   0.132992883 -1.52734697 1.79333274 1.0000000
NH:second:L-CT:second:H   0.205878199 -1.45446165 1.86621805 1.0000000
BSA:third:L-CT:second:H   0.390621030 -1.26971882 2.05096088 0.9999903
CT:third:L-CT:second:H    0.461733222 -1.19860663 2.12207308 0.9998997
NH:third:L-CT:second:H    0.061270823 -1.59906903 1.72161068 1.0000000
BSA:third:H-NH:second:H  -0.169392884 -1.82973274 1.49094697 1.0000000
CT:third:H-NH:second:H   -0.214695877 -1.87503573 1.44564398 1.0000000
NH:third:H-NH:second:H    1.102084361 -0.55825549 2.76242421 0.6087216
BSA:first:L-NH:second:H   0.625529852 -1.03481000 2.28586971 0.9956645
CT:first:L-NH:second:H    0.335311696 -1.32502816 1.99565155 0.9999990
NH:first:L-NH:second:H    0.034490678 -1.62584918 1.69483053 1.0000000
BSA:second:L-NH:second:H -0.081686315 -1.74202617 1.57865354 1.0000000
CT:second:L-NH:second:H  -0.396794698 -2.05713455 1.26354516 0.9999879
NH:second:L-NH:second:H  -0.323909382 -1.98424924 1.33643047 0.9999994
BSA:third:L-NH:second:H  -0.139166551 -1.79950640 1.52117330 1.0000000
CT:third:L-NH:second:H   -0.068054358 -1.72839421 1.59228549 1.0000000
NH:third:L-NH:second:H   -0.468516758 -2.12885661 1.19182310 0.9998780
CT:third:H-BSA:third:H   -0.045302993 -1.70564285 1.61503686 1.0000000
NH:third:H-BSA:third:H    1.271477245 -0.38886261 2.93181710 0.3538770
BSA:first:L-BSA:third:H   0.794922736 -0.86541712 2.45526259 0.9533824
CT:first:L-BSA:third:H    0.504704579 -1.15563527 2.16504443 0.9996750
NH:first:L-BSA:third:H    0.203883561 -1.45645629 1.86422341 1.0000000
BSA:second:L-BSA:third:H  0.087706569 -1.57263328 1.74804642 1.0000000
CT:second:L-BSA:third:H  -0.227401814 -1.88774167 1.43293804 1.0000000
NH:second:L-BSA:third:H  -0.154516499 -1.81485635 1.50582335 1.0000000
BSA:third:L-BSA:third:H   0.030226332 -1.63011352 1.69056619 1.0000000
CT:third:L-BSA:third:H    0.101338525 -1.55900133 1.76167838 1.0000000
NH:third:L-BSA:third:H   -0.299123874 -1.95946373 1.36121598 0.9999998
NH:third:H-CT:third:H     1.316780239 -0.34355961 2.97712009 0.2954817
BSA:first:L-CT:third:H    0.840225729 -0.82011412 2.50056558 0.9263979
CT:first:L-CT:third:H     0.550007573 -1.11033228 2.21034743 0.9990405
NH:first:L-CT:third:H     0.249186555 -1.41115330 1.90952641 1.0000000
BSA:second:L-CT:third:H   0.133009562 -1.52733029 1.79334942 1.0000000
CT:second:L-CT:third:H   -0.182098821 -1.84243867 1.47824103 1.0000000
NH:second:L-CT:third:H   -0.109213505 -1.76955336 1.55112635 1.0000000
BSA:third:L-CT:third:H    0.075529326 -1.58481053 1.73586918 1.0000000
CT:third:L-CT:third:H     0.146641519 -1.51369833 1.80698137 1.0000000
NH:third:L-CT:third:H    -0.253820881 -1.91416073 1.40651897 1.0000000
BSA:first:L-NH:third:H   -0.476554509 -2.13689436 1.18378534 0.9998469
CT:first:L-NH:third:H    -0.766772666 -2.42711252 0.89356719 0.9660421
NH:first:L-NH:third:H    -1.067593684 -2.72793354 0.59274617 0.6619560
BSA:second:L-NH:third:H  -1.183770676 -2.84411053 0.47656918 0.4813148
CT:second:L-NH:third:H   -1.498879059 -3.15921891 0.16146079 0.1251657
NH:second:L-NH:third:H   -1.425993744 -3.08633360 0.23434611 0.1808846
BSA:third:L-NH:third:H   -1.241250913 -2.90159077 0.41908894 0.3959387
CT:third:L-NH:third:H    -1.170138720 -2.83047857 0.49020113 0.5023167
NH:third:L-NH:third:H    -1.570601119 -3.23094097 0.08973873 0.0846379
CT:first:L-BSA:first:L   -0.290218156 -1.95055801 1.37012170 0.9999999
NH:first:L-BSA:first:L   -0.591039174 -2.25137903 1.06930068 0.9977298
BSA:second:L-BSA:first:L -0.707216167 -2.36755602 0.95312369 0.9841937
CT:second:L-BSA:first:L  -1.022324550 -2.68266440 0.63801530 0.7288342
NH:second:L-BSA:first:L  -0.949439234 -2.60977909 0.71090062 0.8241562
BSA:third:L-BSA:first:L  -0.764696403 -2.42503626 0.89564345 0.9668622
CT:third:L-BSA:first:L   -0.693584210 -2.35392406 0.96675564 0.9869917
NH:third:L-BSA:first:L   -1.094046610 -2.75438646 0.56629324 0.6212380
NH:first:L-CT:first:L    -0.300821018 -1.96116087 1.35951884 0.9999998
BSA:second:L-CT:first:L  -0.416998011 -2.07733786 1.24334184 0.9999754
CT:second:L-CT:first:L   -0.732106393 -2.39244625 0.92823346 0.9778795
```



```
NH:second:L-CT:first:L    -0.659221078 -2.31956093 1.00111878 0.9923224
BSA:third:L-CT:first:L    -0.474478247 -2.13481810 1.18586161 0.9998555
CT:third:L-CT:first:L     -0.403366054 -2.06370591 1.25697380 0.9999847
NH:third:L-CT:first:L     -0.803828454 -2.46416831 0.85651140 0.9487473
BSA:second:L-NH:first:L   -0.116176993 -1.77651685 1.54416286 1.0000000
CT:second:L-NH:first:L    -0.431285375 -2.09162523 1.22905448 0.9999606
NH:second:L-NH:first:L    -0.358400060 -2.01873991 1.30193979 0.9999972
BSA:third:L-NH:first:L    -0.173657229 -1.83399708 1.48668262 1.0000000
CT:third:L-NH:first:L     -0.102545036 -1.76288489 1.55779482 1.0000000
NH:third:L-NH:first:L     -0.503007436 -2.16334729 1.15733242 0.9996889
CT:second:L-BSA:second:L  -0.315108383 -1.97544824 1.34523147 0.9999996
NH:second:L-BSA:second:L  -0.242223067 -1.90256292 1.41811679 1.0000000
BSA:third:L-BSA:second:L  -0.057480236 -1.71782009 1.60285962 1.0000000
CT:third:L-BSA:second:L    0.013631957 -1.64670790 1.67397181 1.0000000
NH:third:L-BSA:second:L   -0.386830443 -2.04717030 1.27350941 0.9999916
NH:second:L-CT:second:L    0.072885315 -1.58745454 1.73322517 1.0000000
BSA:third:L-CT:second:L    0.257628146 -1.40271171 1.91796800 1.0000000
CT:third:L-CT:second:L     0.328740339 -1.33159951 1.98908019 0.9999992
NH:third:L-CT:second:L    -0.071722060 -1.73206191 1.58861779 1.0000000
BSA:third:L-NH:second:L    0.184742831 -1.47559702 1.84508268 1.0000000
CT:third:L-NH:second:L     0.255855024 -1.40448483 1.91619488 1.0000000
NH:third:L-NH:second:L    -0.144607376 -1.80494723 1.51573248 1.0000000
CT:third:L-BSA:third:L     0.071112193 -1.58922766 1.73145205 1.0000000
NH:third:L-BSA:third:L    -0.329350207 -1.98969006 1.33098965 0.9999992
NH:third:L-CT:third:L     -0.400462400 -2.06080225 1.25987745 0.9999862
```

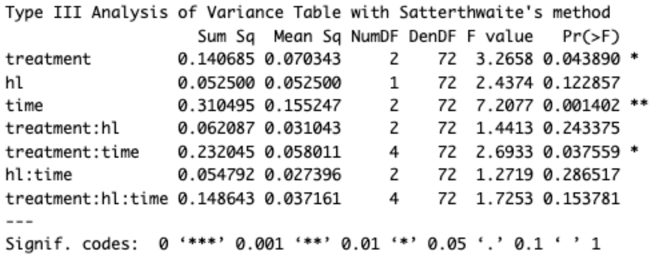
Figure 32. Tukey test of MnP activity in Marasmius, interactions time, medium and concentration.

Marasmius peptidase



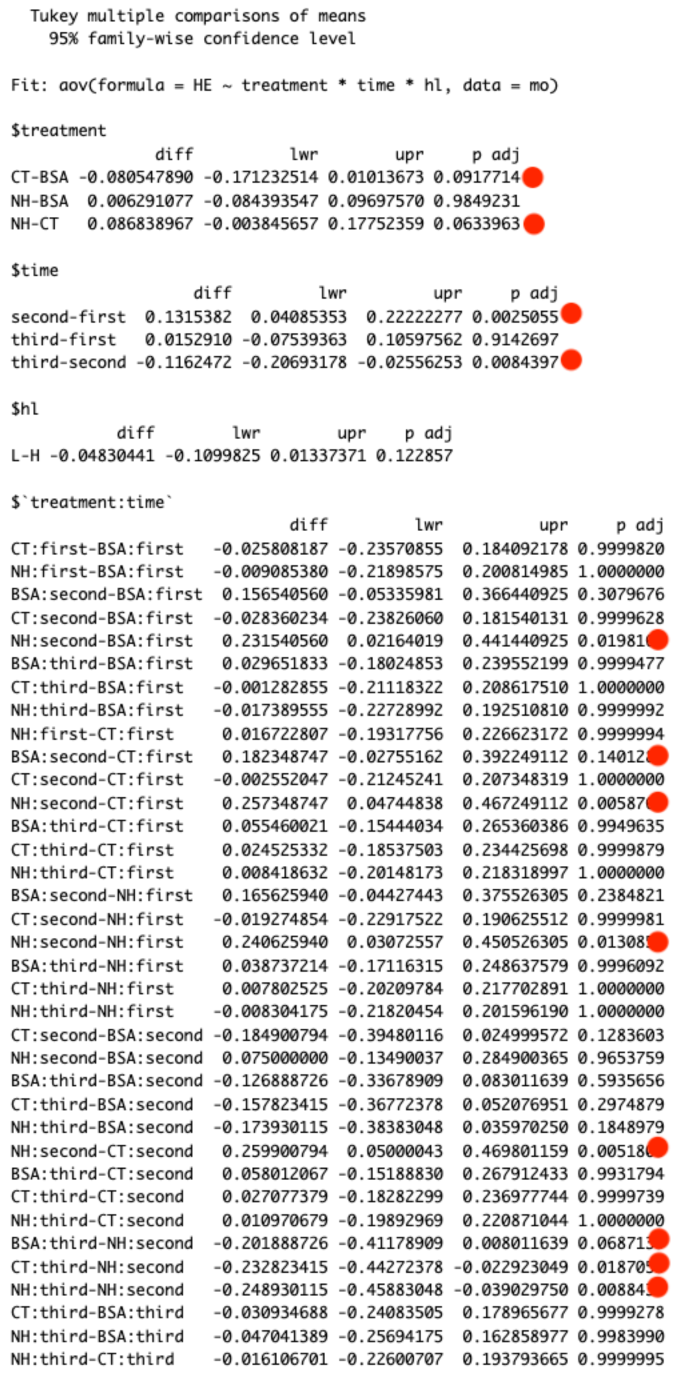
```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: HE ~ treatment * hl * time + (1 | ID)
   Data: mo

REML criterion at convergence: -43

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.9653 -0.1784 -0.0282  0.1018  5.0649

Random effects:
 Groups   Name        Variance Std.Dev.
 ID       (Intercept) 0.00000  0.0000
 Residual             0.02154  0.1468
Number of obs: 90, groups:  ID, 30

Fixed effects:
                             Estimate Std. Error         df t value Pr(>|t|)
(Intercept)                 0.0297887  0.0656340 72.0000000   0.454   0.6513
treatmentCT                -0.0256468  0.0928205 72.0000000  -0.276   0.7831
treatmentNH                -0.0048811  0.0928205 72.0000000  -0.053   0.9582
hlL                        -0.0028569  0.0928205 72.0000000  -0.031   0.9755
timesecond                  0.1560446  0.0928205 72.0000000   1.681   0.0971 .
timethird                   0.0603690  0.0928205 72.0000000   0.650   0.5175
treatmentCT:hlL            -0.0003228  0.1312681 72.0000000  -0.002   0.9980
treatmentNH:hlL            -0.0084086  0.1312681 72.0000000  -0.064   0.9491
treatmentCT:timesecond     -0.1601865  0.1312681 72.0000000  -1.220   0.2263
treatmentNH:timesecond      0.2542462  0.1312681 72.0000000   1.937   0.0567 .
treatmentCT:timethird      -0.0383270  0.1312681 72.0000000  -0.292   0.7711
treatmentNH:timethird      -0.0730696  0.1312681 72.0000000  -0.557   0.5795
hlL:timesecond              0.0009918  0.1312681 72.0000000   0.008   0.9940
hlL:timethird              -0.0614344  0.1312681 72.0000000  -0.468   0.6412
treatmentCT:hlL:timesecond  0.0021879  0.1856411 72.0000000   0.012   0.9906
treatmentNH:hlL:timesecond -0.3403216  0.1856411 72.0000000  -1.833   0.0709 .
treatmentCT:hlL:timethird   0.0664009  0.1856411 72.0000000   0.358   0.7216
treatmentNH:hlL:timethird   0.0702271  0.1856411 72.0000000   0.378   0.7063
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation matrix not shown by default, as p = 18 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it

optimizer (nloptwrap) convergence code: 0 (OK)
boundary (singular) fit: see help('isSingular')
```

Figure 33. Linear mixed model of peptidase activity in Marasmius, interactions time, medium and concentration.

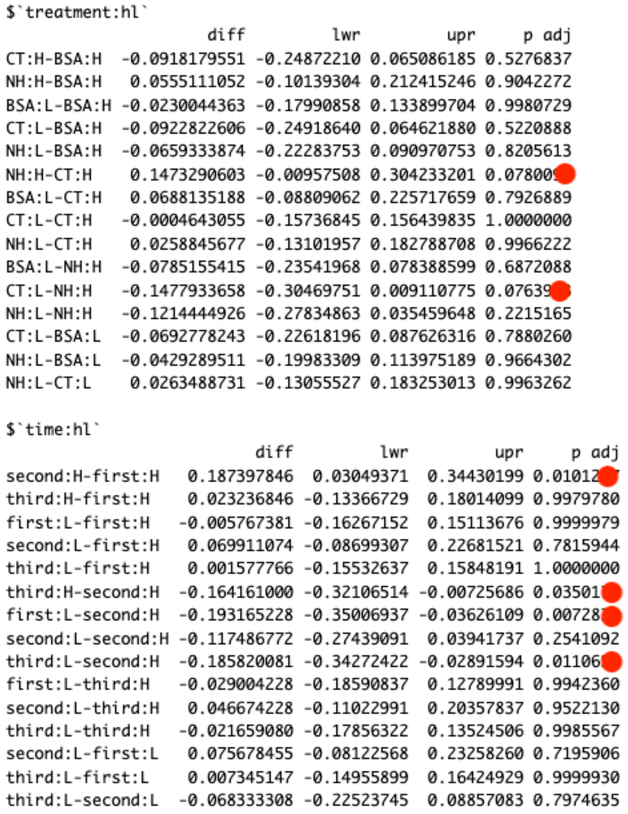


```
Type III Analysis of Variance Table with Satterthwaite's method
                    Sum Sq  Mean Sq NumDF DenDF F value   Pr(>F)
treatment         0.140685 0.070343     2    72  3.2658 0.043890 *
hl                0.052500 0.052500     1    72  2.4374 0.122857
time              0.310495 0.155247     2    72  7.2077 0.001402 **
treatment:hl      0.062087 0.031043     2    72  1.4413 0.243375
treatment:time    0.232045 0.058011     4    72  2.6933 0.037559 *
hl:time           0.054792 0.027396     2    72  1.2719 0.286517
treatment:hl:time 0.148643 0.037161     4    72  1.7253 0.153781
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

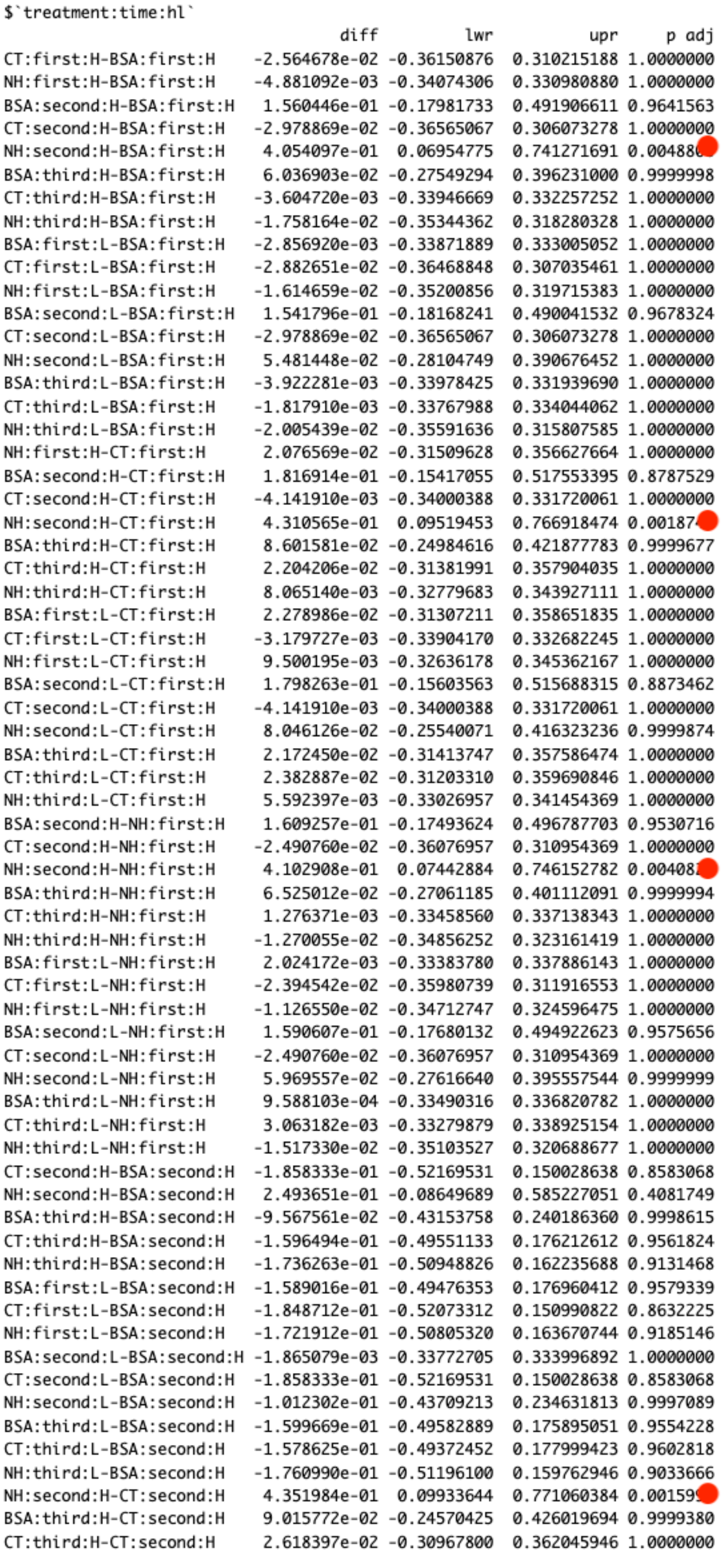
Figure 34. ANOVA of peptidase activity in Marasmius, interactions time, medium and concentration.



```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = HE ~ treatment * time * hl, data = mo)

$treatment
              diff          lwr        upr     p adj
CT-BSA -0.080547890 -0.171232514 0.01013673 0.0917714●
NH-BSA  0.006291077 -0.084393547 0.09697570 0.9849231
NH-CT   0.086838967 -0.003845657 0.17752359 0.0633963●

$time
                   diff         lwr         upr     p adj
second-first  0.1315382  0.04085353  0.22222277 0.0025055●
third-first   0.0152910 -0.07539363  0.10597562 0.9142697
third-second -0.1162472 -0.20693178 -0.02556253 0.0084397●

$hl
           diff        lwr        upr    p adj
L-H -0.04830441 -0.1099825 0.01337371 0.122857

$`treatment:time`
                               diff         lwr          upr     p adj
CT:first-BSA:first    -0.025808187 -0.23570855  0.184092178 0.9999820
NH:first-BSA:first    -0.009085380 -0.21898575  0.200814985 1.0000000
BSA:second-BSA:first   0.156540560 -0.05335981  0.366440925 0.3079676
CT:second-BSA:first   -0.028360234 -0.23826060  0.181540131 0.9999628
NH:second-BSA:first    0.231540560  0.02164019  0.441440925 0.01981●
BSA:third-BSA:first    0.029651833 -0.18024853  0.239552199 0.9999477
CT:third-BSA:first    -0.001282855 -0.21118322  0.208617510 1.0000000
NH:third-BSA:first    -0.017389555 -0.22728992  0.192510810 0.9999992
NH:first-CT:first      0.016722807 -0.19317756  0.226623172 0.9999994
BSA:second-CT:first    0.182348747 -0.02755162  0.392249112 0.14012●
CT:second-CT:first    -0.002552047 -0.21245241  0.207348319 1.0000000
NH:second-CT:first     0.257348747  0.04744838  0.467249112 0.00587●
BSA:third-CT:first     0.055460021 -0.15444034  0.265360386 0.9949635
CT:third-CT:first      0.024525332 -0.18537503  0.234425698 0.9999879
NH:third-CT:first      0.008418632 -0.20148173  0.218318997 1.0000000
BSA:second-NH:first    0.165625940 -0.04427443  0.375526305 0.2384821
CT:second-NH:first    -0.019274854 -0.22917522  0.190625512 0.9999981
NH:second-NH:first     0.240625940  0.03072557  0.450526305 0.01308●
BSA:third-NH:first     0.038737214 -0.17116315  0.248637579 0.9996092
CT:third-NH:first      0.007802525 -0.20209784  0.217702891 1.0000000
NH:third-NH:first     -0.008304175 -0.21820454  0.201596190 1.0000000
CT:second-BSA:second  -0.184900794 -0.39480116  0.024999572 0.1283603
NH:second-BSA:second   0.075000000 -0.13490037  0.284900365 0.9653759
BSA:third-BSA:second  -0.126888726 -0.33678909  0.083011639 0.5935656
CT:third-BSA:second   -0.157823415 -0.36772378  0.052076951 0.2974879
NH:third-BSA:second   -0.173930115 -0.38383048  0.035970250 0.1848979
NH:second-CT:second    0.259900794  0.05000043  0.469801159 0.00518●
BSA:third-CT:second    0.058012067 -0.15188830  0.267912433 0.9931794
CT:third-CT:second     0.027077379 -0.18282299  0.236977744 0.9999739
NH:third-CT:second     0.010970679 -0.19892969  0.220871044 1.0000000
BSA:third-NH:second   -0.201888726 -0.41178909  0.008011639 0.06871●
CT:third-NH:second    -0.232823415 -0.44272378 -0.022923049 0.01870●
NH:third-NH:second    -0.248930115 -0.45883048 -0.039029750 0.00884●
CT:third-BSA:third    -0.030934688 -0.24083505  0.178965677 0.9999278
NH:third-BSA:third    -0.047041389 -0.25694175  0.162858977 0.9983990
NH:third-CT:third     -0.016106701 -0.22600707  0.193793665 0.9999995
```

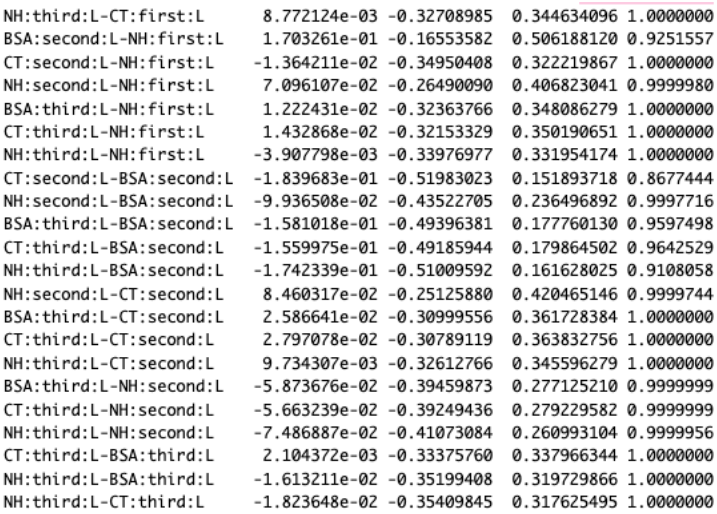


```
$`treatment:hl`
                      diff         lwr         upr     p adj
CT:H-BSA:H   -0.0918179551 -0.24872210 0.065086185 0.5276837
NH:H-BSA:H    0.0555111052 -0.10139304 0.212415246 0.9042272
BSA:L-BSA:H -0.0230044363 -0.17990858 0.133899704 0.9980729
CT:L-BSA:H   -0.0922822606 -0.24918640 0.064621880 0.5220888
NH:L-BSA:H   -0.0659333874 -0.22283753 0.090970753 0.8205613
NH:H-CT:H     0.1473290603 -0.00957508 0.304233201 0.07800
BSA:L-CT:H    0.0688135188 -0.08809062 0.225717659 0.7926889
CT:L-CT:H    -0.0004643055 -0.15736845 0.156439835 1.0000000
NH:L-CT:H     0.0258845677 -0.13101957 0.182788708 0.9966222
BSA:L-NH:H   -0.0785155415 -0.23541968 0.078388599 0.6872088
CT:L-NH:H    -0.1477933658 -0.30469751 0.009110775 0.07639
NH:L-NH:H    -0.1214444926 -0.27834863 0.035459648 0.2215165
CT:L-BSA:L   -0.0692778243 -0.22618196 0.087626316 0.7880260
NH:L-BSA:L   -0.0429289511 -0.19983309 0.113975189 0.9664302
NH:L-CT:L     0.0263488731 -0.13055527 0.183253013 0.9963262

$`time:hl`
                           diff         lwr         upr     p adj
second:H-first:H    0.187397846  0.03049371  0.34430199 0.01012
third:H-first:H     0.023236846 -0.13366729  0.18014099 0.9979780
first:L-first:H    -0.005767381 -0.16267152  0.15113676 0.9999979
second:L-first:H    0.069911074 -0.08699307  0.22681521 0.7815944
third:L-first:H     0.001577766 -0.15532637  0.15848191 1.0000000
third:H-second:H   -0.164161000 -0.32106514 -0.00725686 0.03501
first:L-second:H   -0.193165228 -0.35006937 -0.03626109 0.00728
second:L-second:H -0.117486772 -0.27439091  0.03941737 0.2541092
third:L-second:H   -0.185820081 -0.34272422 -0.02891594 0.01106
first:L-third:H    -0.029004228 -0.18590837  0.12789991 0.9942360
second:L-third:H    0.046674228 -0.11022991  0.20357837 0.9522130
third:L-third:H    -0.021659080 -0.17856322  0.13524506 0.9985567
second:L-first:L    0.075678455 -0.08122568  0.23258260 0.7195906
third:L-first:L     0.007345147 -0.14955899  0.16424929 0.9999930
third:L-second:L   -0.068333308 -0.22523745  0.08857083 0.7974635
```



```
$`treatment:time:hl`
                                  diff          lwr         upr     p adj
CT:first:H-BSA:first:H     -2.564678e-02 -0.36150876  0.310215188 1.0000000
NH:first:H-BSA:first:H     -4.881092e-03 -0.34074306  0.330980880 1.0000000
BSA:second:H-BSA:first:H    1.560446e-01 -0.17981733  0.491906611 0.9641563
CT:second:H-BSA:first:H    -2.978869e-02 -0.36565067  0.306073278 1.0000000
NH:second:H-BSA:first:H     4.054097e-01  0.06954775  0.741271691 0.004886[illegible]
BSA:third:H-BSA:first:H     6.036903e-02 -0.27549294  0.396231000 0.9999998
CT:third:H-BSA:first:H     -3.604720e-03 -0.33946669  0.332257252 1.0000000
NH:third:H-BSA:first:H     -1.758164e-02 -0.35344362  0.318280328 1.0000000
BSA:first:L-BSA:first:H    -2.856920e-03 -0.33871889  0.333005052 1.0000000
CT:first:L-BSA:first:H     -2.882651e-02 -0.36468848  0.307035461 1.0000000
NH:first:L-BSA:first:H     -1.614659e-02 -0.35200856  0.319715383 1.0000000
BSA:second:L-BSA:first:H    1.541796e-01 -0.18168241  0.490041532 0.9678324
CT:second:L-BSA:first:H    -2.978869e-02 -0.36565067  0.306073278 1.0000000
NH:second:L-BSA:first:H     5.481448e-02 -0.28104749  0.390676452 1.0000000
BSA:third:L-BSA:first:H    -3.922281e-03 -0.33978425  0.331939690 1.0000000
CT:third:L-BSA:first:H     -1.817910e-03 -0.33767988  0.334044062 1.0000000
NH:third:L-BSA:first:H     -2.005439e-02 -0.35591636  0.315807585 1.0000000
NH:first:H-CT:first:H       2.076569e-02 -0.31509628  0.356627664 1.0000000
BSA:second:H-CT:first:H     1.816914e-01 -0.15417055  0.517553395 0.8787529
CT:second:H-CT:first:H     -4.141910e-03 -0.34000388  0.331720061 1.0000000
NH:second:H-CT:first:H      4.310565e-01  0.09519453  0.766918474 0.001874[illegible]
BSA:third:H-CT:first:H      8.601581e-02 -0.24984616  0.421877783 0.9999677
CT:third:H-CT:first:H       2.204206e-02 -0.31381991  0.357904035 1.0000000
NH:third:H-CT:first:H       8.065140e-03 -0.32779683  0.343927111 1.0000000
BSA:first:L-CT:first:H      2.278986e-02 -0.31307211  0.358651835 1.0000000
CT:first:L-CT:first:H      -3.179727e-03 -0.33904170  0.332682245 1.0000000
NH:first:L-CT:first:H       9.500195e-03 -0.32636178  0.345362167 1.0000000
BSA:second:L-CT:first:H     1.798263e-01 -0.15603563  0.515688315 0.8873462
CT:second:L-CT:first:H     -4.141910e-03 -0.34000388  0.331720061 1.0000000
NH:second:L-CT:first:H      8.046126e-02 -0.25540071  0.416323236 0.9999874
BSA:third:L-CT:first:H      2.172450e-02 -0.31413747  0.357586474 1.0000000
CT:third:L-CT:first:H       2.382887e-02 -0.31203310  0.359690846 1.0000000
NH:third:L-CT:first:H       5.592397e-03 -0.33026957  0.341454369 1.0000000
BSA:second:H-NH:first:H     1.609257e-01 -0.17493624  0.496787703 0.9530716
CT:second:H-NH:first:H     -2.490760e-02 -0.36076957  0.310954369 1.0000000
NH:second:H-NH:first:H      4.102908e-01  0.07442884  0.746152782 0.004082[illegible]
BSA:third:H-NH:first:H      6.525012e-02 -0.27061185  0.401112091 0.9999994
CT:third:H-NH:first:H       1.276371e-03 -0.33458560  0.337138343 1.0000000
NH:third:H-NH:first:H      -1.270055e-02 -0.34856252  0.323161419 1.0000000
BSA:first:L-NH:first:H      2.024172e-03 -0.33383780  0.337886143 1.0000000
CT:first:L-NH:first:H      -2.394542e-02 -0.35980739  0.311916553 1.0000000
NH:first:L-NH:first:H      -1.126550e-02 -0.34712747  0.324596475 1.0000000
BSA:second:L-NH:first:H     1.590607e-01 -0.17680132  0.494922623 0.9575656
CT:second:L-NH:first:H     -2.490760e-02 -0.36076957  0.310954369 1.0000000
NH:second:L-NH:first:H      5.969557e-02 -0.27616640  0.395557544 0.9999999
BSA:third:L-NH:first:H      9.588103e-04 -0.33490316  0.336820782 1.0000000
CT:third:L-NH:first:H       3.063182e-03 -0.33279879  0.338925154 1.0000000
NH:third:L-NH:first:H      -1.517330e-02 -0.35103527  0.320688677 1.0000000
CT:second:H-BSA:second:H   -1.858333e-01 -0.52169531  0.150028638 0.8583068
NH:second:H-BSA:second:H    2.493651e-01 -0.08649689  0.585227051 0.4081749
BSA:third:H-BSA:second:H   -9.567561e-02 -0.43153758  0.240186360 0.9998615
CT:third:H-BSA:second:H    -1.596494e-01 -0.49551133  0.176212612 0.9561824
NH:third:H-BSA:second:H    -1.736263e-01 -0.50948826  0.162235688 0.9131468
BSA:first:L-BSA:second:H   -1.589016e-01 -0.49476353  0.176960412 0.9579339
CT:first:L-BSA:second:H    -1.848712e-01 -0.52073312  0.150990822 0.8632225
NH:first:L-BSA:second:H    -1.721912e-01 -0.50805320  0.163670744 0.9185146
BSA:second:L-BSA:second:H -1.865079e-03 -0.33772705  0.333996892 1.0000000
CT:second:L-BSA:second:H   -1.858333e-01 -0.52169531  0.150028638 0.8583068
NH:second:L-BSA:second:H   -1.012302e-01 -0.43709213  0.234631813 0.9997089
BSA:third:L-BSA:second:H   -1.599669e-01 -0.49582889  0.175895051 0.9554228
CT:third:L-BSA:second:H    -1.578625e-01 -0.49372452  0.177999423 0.9602818
NH:third:L-BSA:second:H    -1.760990e-01 -0.51196100  0.159762946 0.9033666
NH:second:H-CT:second:H     4.351984e-01  0.09933644  0.771060384 0.001599[illegible]
BSA:third:H-CT:second:H     9.015772e-02 -0.24570425  0.426019694 0.9999380
CT:third:H-CT:second:H      2.618397e-02 -0.30967800  0.362045946 1.0000000
```

```
NH:third:H-CT:second:H      1.220705e-02 -0.32365492  0.348069022 1.0000000
BSA:first:L-CT:second:H     2.693177e-02 -0.30893020  0.362793746 1.0000000
CT:first:L-CT:second:H      9.621832e-04 -0.33489979  0.336824155 1.0000000
NH:first:L-CT:second:H      1.364211e-02 -0.32221987  0.349504077 1.0000000
BSA:second:L-CT:second:H    1.839683e-01 -0.15189372  0.519830226 0.8677444
CT:second:L-CT:second:H    -1.734723e-16 -0.33586197  0.335861972 1.0000000
NH:second:L-CT:second:H     8.460317e-02 -0.25125880  0.420465146 0.9999744
BSA:third:L-CT:second:H     2.586641e-02 -0.30999556  0.361728384 1.0000000
CT:third:L-CT:second:H      2.797078e-02 -0.30789119  0.363832756 1.0000000
NH:third:L-CT:second:H      9.734307e-03 -0.32612766  0.345596279 1.0000000
BSA:third:H-NH:second:H    -3.450407e-01 -0.68090266 -0.009178719 0.03777●
CT:third:H-NH:second:H     -4.090144e-01 -0.74487641 -0.073152467 0.00427●
NH:third:H-NH:second:H     -4.229914e-01 -0.75885333 -0.087129391 0.00254●
BSA:first:L-NH:second:H    -4.082666e-01 -0.74412861 -0.072404667 0.00439●
CT:first:L-NH:second:H     -4.342362e-01 -0.77009820 -0.098374258 0.00166●
NH:first:L-NH:second:H     -4.215563e-01 -0.75741828 -0.085694336 0.00268●
BSA:second:L-NH:second:H   -2.512302e-01 -0.58709213  0.084631813 0.3949252
CT:second:L-NH:second:H    -4.351984e-01 -0.77106038 -0.099336441 0.00159●
NH:second:L-NH:second:H    -3.505952e-01 -0.68645721 -0.014733266 0.03173●
BSA:third:L-NH:second:H    -4.093320e-01 -0.74519397 -0.073470028 0.00422●
CT:third:L-NH:second:H     -4.072276e-01 -0.74308960 -0.071365657 0.00456●
NH:third:L-NH:second:H     -4.254641e-01 -0.76132608 -0.089602134 0.00231●
CT:third:H-BSA:third:H     -6.397375e-02 -0.39983572  0.271888223 0.9999996
NH:third:H-BSA:third:H     -7.795067e-02 -0.41381264  0.257911300 0.9999920
BSA:first:L-BSA:third:H    -6.322595e-02 -0.39908792  0.272636024 0.9999996
CT:first:L-BSA:third:H     -8.919554e-02 -0.42505751  0.246666433 0.9999465
NH:first:L-BSA:third:H     -7.651562e-02 -0.41237759  0.259346355 0.9999939
BSA:second:L-BSA:third:H    9.381053e-02 -0.24205144  0.429672504 0.9998937
CT:second:L-BSA:third:H    -9.015772e-02 -0.42601969  0.245704250 0.9999380
NH:second:L-BSA:third:H    -5.554547e-03 -0.34141652  0.330307424 1.0000000
BSA:third:L-BSA:third:H    -6.429131e-02 -0.40015328  0.271570662 0.9999995
CT:third:L-BSA:third:H     -6.218694e-02 -0.39804891  0.273675034 0.9999997
NH:third:L-BSA:third:H     -8.042341e-02 -0.41628539  0.255438557 0.9999875
NH:third:H-CT:third:H      -1.397692e-02 -0.34983890  0.321885048 1.0000000
BSA:first:L-CT:third:H      7.478001e-04 -0.33511417  0.336609772 1.0000000
CT:first:L-CT:third:H      -2.522179e-02 -0.36108376  0.310640181 1.0000000
NH:first:L-CT:third:H      -1.254187e-02 -0.34840384  0.323320103 1.0000000
BSA:second:L-CT:third:H     1.577843e-01 -0.17807769  0.493646252 0.9604547
CT:second:L-CT:third:H     -2.618397e-02 -0.36204595  0.309677998 1.0000000
NH:second:L-CT:third:H      5.841920e-02 -0.27744277  0.394281173 0.9999999
BSA:third:L-CT:third:H     -3.175611e-04 -0.33617953  0.335544411 1.0000000
CT:third:L-CT:third:H       1.786811e-03 -0.33407516  0.337648782 1.0000000
NH:third:L-CT:third:H      -1.644967e-02 -0.35231164  0.319412305 1.0000000
BSA:first:L-NH:third:H      1.472472e-02 -0.32113725  0.350586696 1.0000000
CT:first:L-NH:third:H      -1.124487e-02 -0.34710684  0.324617105 1.0000000
NH:first:L-NH:third:H       1.435055e-03 -0.33442692  0.337297027 1.0000000
BSA:second:L-NH:third:H     1.717612e-01 -0.16410077  0.507623176 0.9200793
CT:second:L-NH:third:H     -1.220705e-02 -0.34806902  0.323654922 1.0000000
NH:second:L-NH:third:H      7.239612e-02 -0.26346585  0.408258097 0.9999973
BSA:third:L-NH:third:H      1.365936e-02 -0.32220261  0.349521335 1.0000000
CT:third:L-NH:third:H       1.576373e-02 -0.32009824  0.351625706 1.0000000
NH:third:L-NH:third:H      -2.472743e-03 -0.33833471  0.333389229 1.0000000
CT:first:L-BSA:first:L     -2.596959e-02 -0.36183156  0.309892381 1.0000000
NH:first:L-BSA:first:L     -1.328967e-02 -0.34915164  0.322572303 1.0000000
BSA:second:L-BSA:first:L    1.570365e-01 -0.17882549  0.492898452 0.9620788
CT:second:L-BSA:first:L    -2.693177e-02 -0.36279375  0.308930198 1.0000000
NH:second:L-BSA:first:L     5.767140e-02 -0.27819057  0.393533372 0.9999999
BSA:third:L-BSA:first:L    -1.065361e-03 -0.33692733  0.334796611 1.0000000
CT:third:L-BSA:first:L      1.039010e-03 -0.33482296  0.336900982 1.0000000
NH:third:L-BSA:first:L     -1.719747e-02 -0.35305944  0.318664505 1.0000000
NH:first:L-CT:first:L       1.267992e-02 -0.32318205  0.348541894 1.0000000
BSA:second:L-CT:first:L     1.830061e-01 -0.15285590  0.518868043 0.8724657
CT:second:L-CT:first:L     -9.621832e-04 -0.33682416  0.334899789 1.0000000
NH:second:L-CT:first:L      8.364099e-02 -0.25222098  0.419502963 0.9999782
BSA:third:L-CT:first:L      2.490423e-02 -0.31095774  0.360766201 1.0000000
CT:third:L-CT:first:L       2.700860e-02 -0.30885337  0.362870573 1.0000000
```

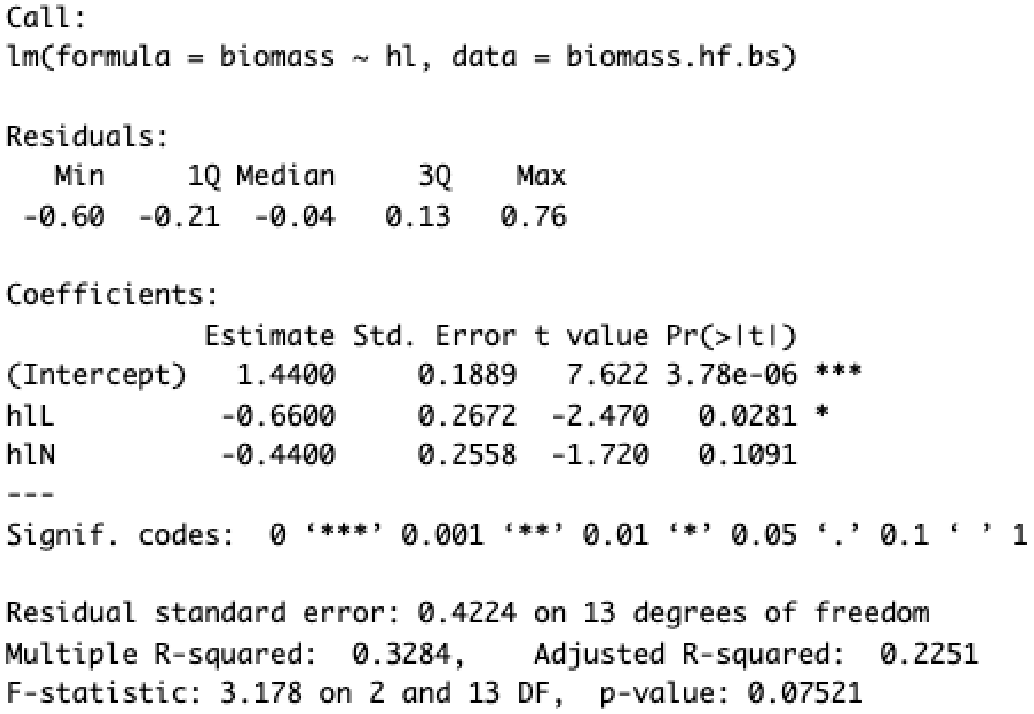


```
NH:third:L-CT:first:L       8.772124e-03 -0.32708985  0.344634096 1.0000000
BSA:second:L-NH:first:L     1.703261e-01 -0.16553582  0.506188120 0.9251557
CT:second:L-NH:first:L     -1.364211e-02 -0.34950408  0.322219867 1.0000000
NH:second:L-NH:first:L      7.096107e-02 -0.26490090  0.406823041 0.9999980
BSA:third:L-NH:first:L      1.222431e-02 -0.32363766  0.348086279 1.0000000
CT:third:L-NH:first:L       1.432868e-02 -0.32153329  0.350190651 1.0000000
NH:third:L-NH:first:L      -3.907798e-03 -0.33976977  0.331954174 1.0000000
CT:second:L-BSA:second:L   -1.839683e-01 -0.51983023  0.151893718 0.8677444
NH:second:L-BSA:second:L   -9.936508e-02 -0.43522705  0.236496892 0.9997716
BSA:third:L-BSA:second:L   -1.581018e-01 -0.49396381  0.177760130 0.9597498
CT:third:L-BSA:second:L    -1.559975e-01 -0.49185944  0.179864502 0.9642529
NH:third:L-BSA:second:L    -1.742339e-01 -0.51009592  0.161628025 0.9108058
NH:second:L-CT:second:L     8.460317e-02 -0.25125880  0.420465146 0.9999744
BSA:third:L-CT:second:L     2.586641e-02 -0.30999556  0.361728384 1.0000000
CT:third:L-CT:second:L      2.797078e-02 -0.30789119  0.363832756 1.0000000
NH:third:L-CT:second:L      9.734307e-03 -0.32612766  0.345596279 1.0000000
BSA:third:L-NH:second:L    -5.873676e-02 -0.39459873  0.277125210 0.9999999
CT:third:L-NH:second:L     -5.663239e-02 -0.39249436  0.279229582 0.9999999
NH:third:L-NH:second:L     -7.486887e-02 -0.41073084  0.260993104 0.9999956
CT:third:L-BSA:third:L      2.104372e-03 -0.33375760  0.337966344 1.0000000
NH:third:L-BSA:third:L     -1.613211e-02 -0.35199408  0.319729866 1.0000000
NH:third:L-CT:third:L      -1.823648e-02 -0.35409845  0.317625495 1.0000000
```

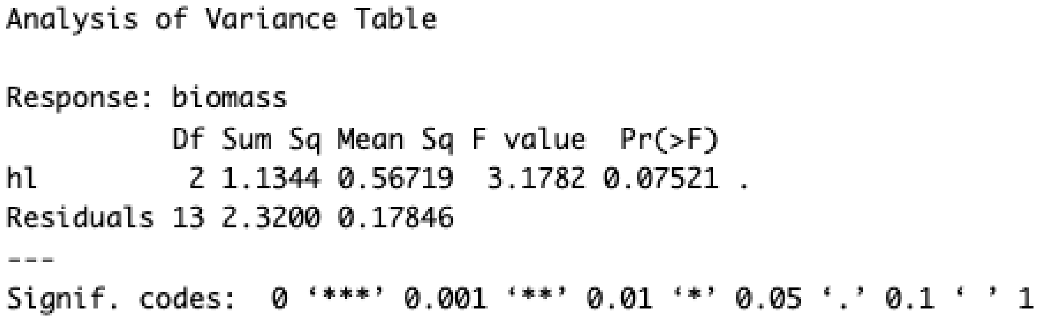
Figure 35. Tukey test of peptidase activity in Marasmius, interactions time, medium and concentration.

Appendix 2

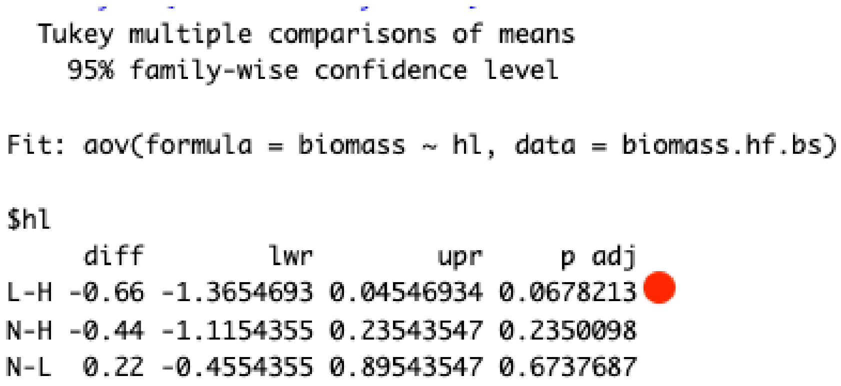
Biomass Hypholoma NH_4*high/low and No N*



```
Call:
lm(formula = biomass ~ hl, data = biomass.hf.bs)

Residuals:
   Min     1Q Median     3Q    Max 
 -0.60  -0.21  -0.04   0.13   0.76 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   1.4400     0.1889   7.622 3.78e-06 ***
hlL          -0.6600     0.2672  -2.470   0.0281 *  
hlN          -0.4400     0.2558  -1.720   0.1091    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4224 on 13 degrees of freedom
Multiple R-squared:  0.3284,	Adjusted R-squared:  0.2251 
F-statistic: 3.178 on 2 and 13 DF,  p-value: 0.07521
```

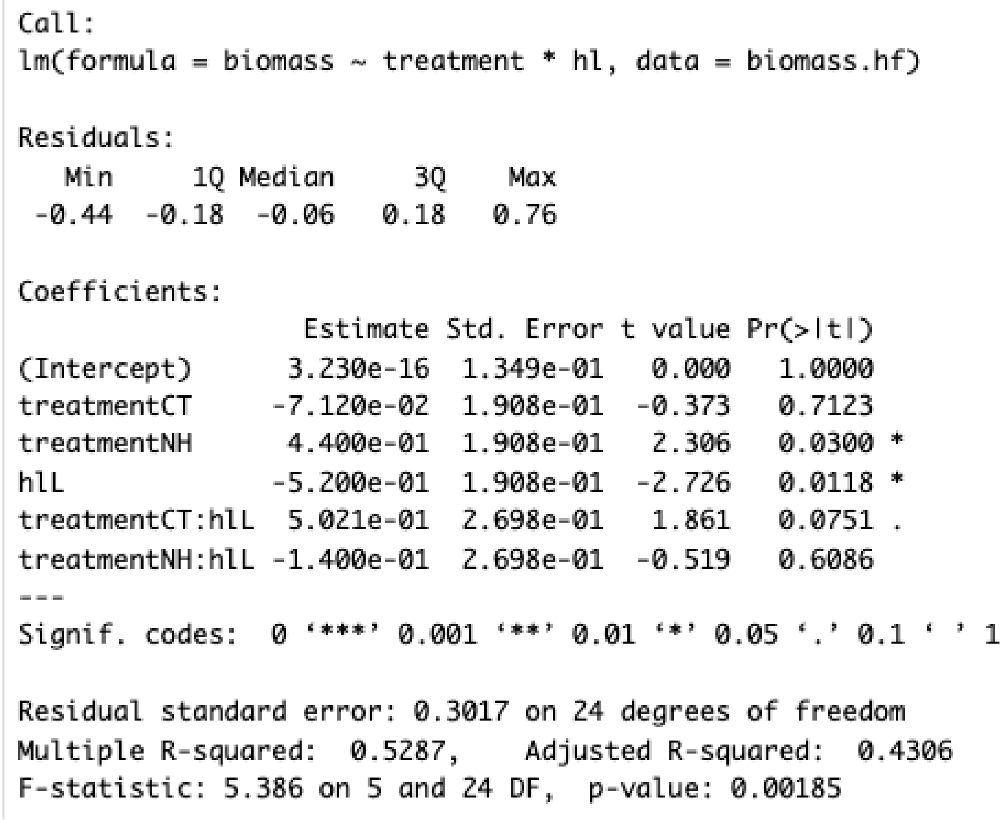


```
Analysis of Variance Table

Response: biomass
          Df Sum Sq Mean Sq F value  Pr(>F)  
hl         2 1.1344 0.56719  3.1782 0.07521 .
Residuals 13 2.3200 0.17846                  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

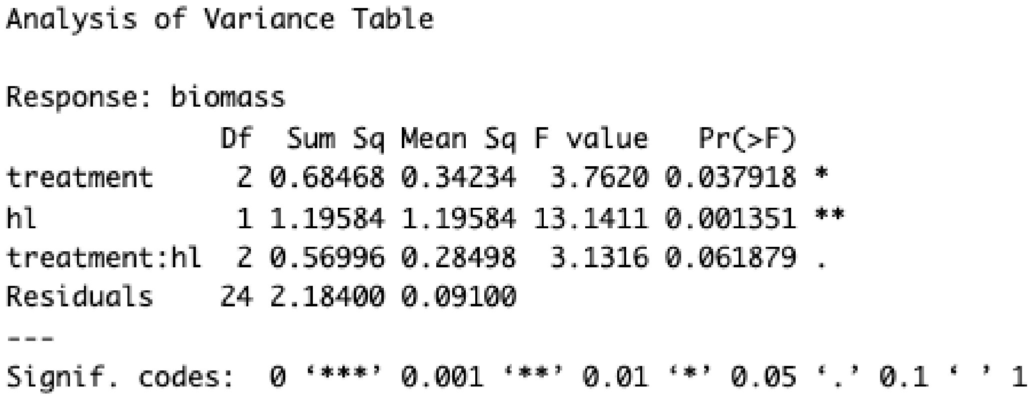


```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = biomass ~ hl, data = biomass.hf.bs)

$hl
     diff        lwr        upr     p adj
L-H -0.66 -1.3654693 0.04546934 0.0678213 ●
N-H -0.44 -1.1154355 0.23543547 0.2350098
N-L  0.22 -0.4554355 0.89543547 0.6737687
```

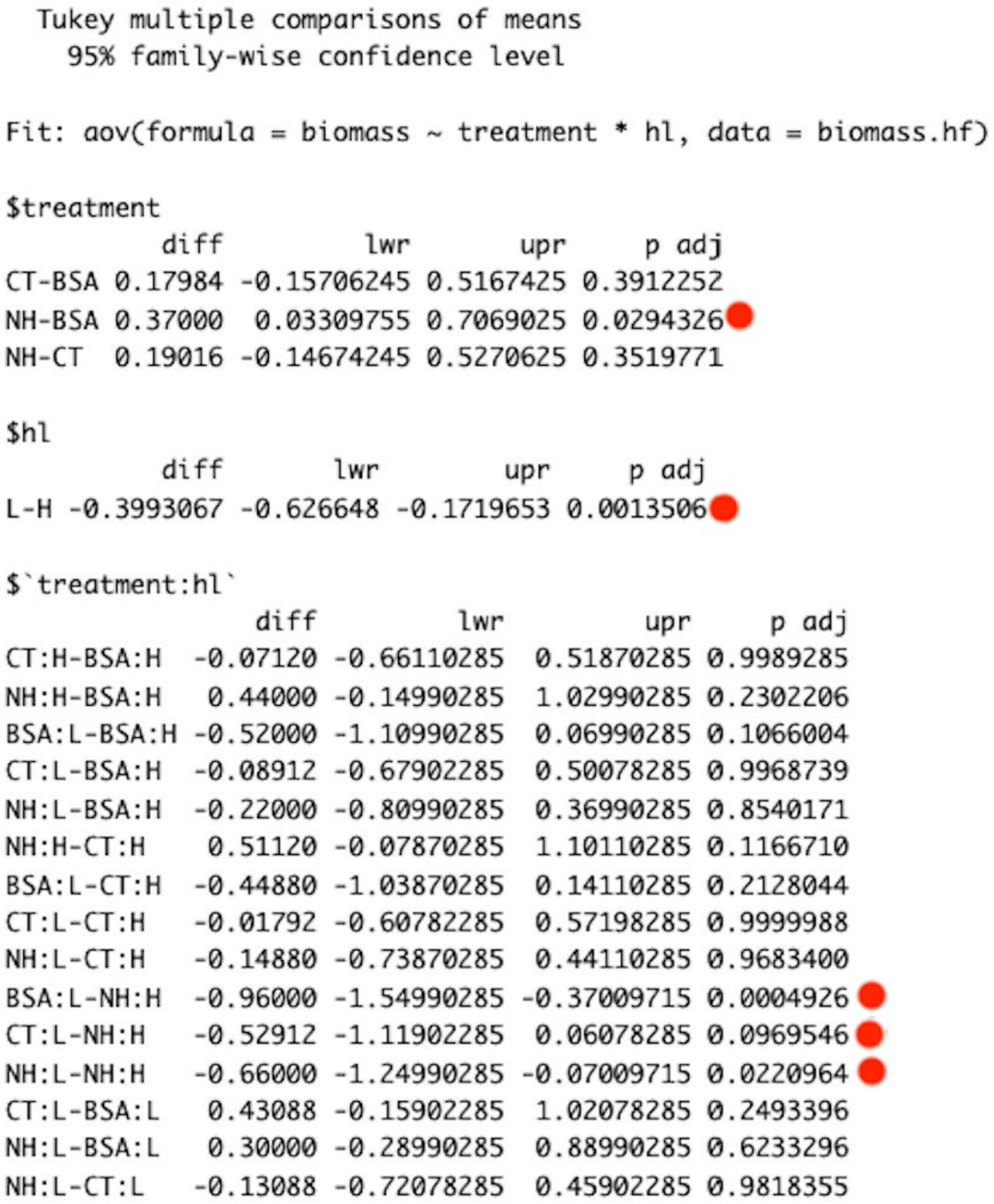
Biomass Hypholoma



```
Call:
lm(formula = biomass ~ treatment * hl, data = biomass.hf)

Residuals:
   Min     1Q Median     3Q    Max 
 -0.44  -0.18  -0.06   0.18   0.76 

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)  
(Intercept)       3.230e-16  1.349e-01   0.000   1.0000  
treatmentCT      -7.120e-02  1.908e-01  -0.373   0.7123  
treatmentNH       4.400e-01  1.908e-01   2.306   0.0300 *
hlL              -5.200e-01  1.908e-01  -2.726   0.0118 *
treatmentCT:hlL   5.021e-01  2.698e-01   1.861   0.0751 .
treatmentNH:hlL  -1.400e-01  2.698e-01  -0.519   0.6086  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3017 on 24 degrees of freedom
Multiple R-squared:  0.5287,	Adjusted R-squared:  0.4306 
F-statistic: 5.386 on 5 and 24 DF,  p-value: 0.00185
```

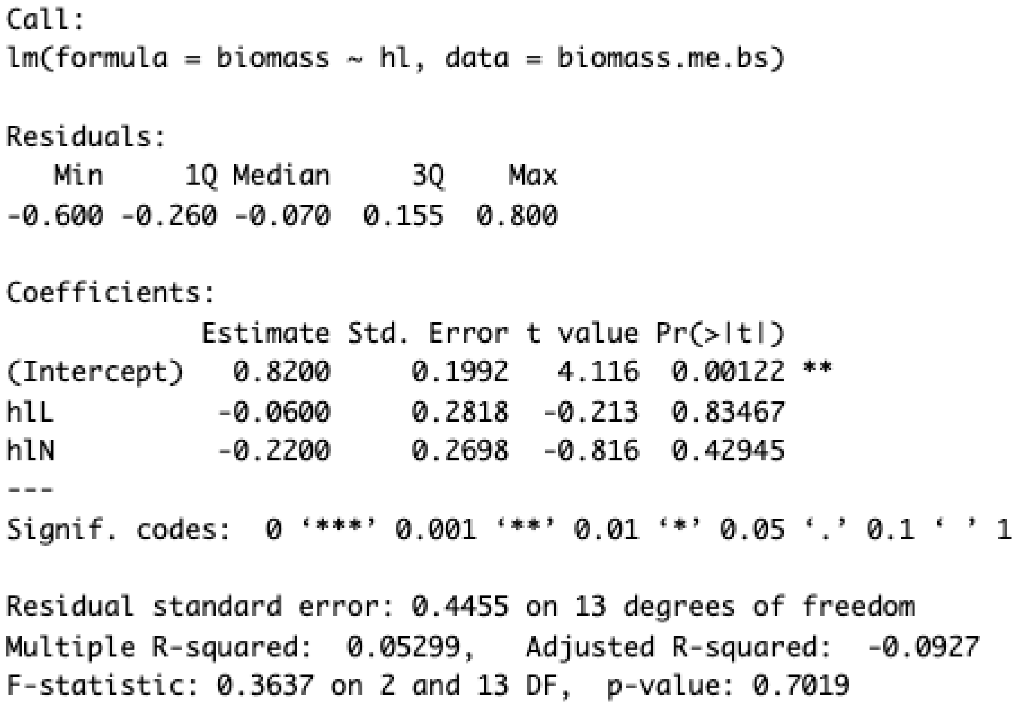


```
Analysis of Variance Table

Response: biomass
             Df  Sum Sq Mean Sq F value   Pr(>F)   
treatment     2 0.68468 0.34234  3.7620 0.037918 * 
hl            1 1.19584 1.19584 13.1411 0.001351 **
treatment:hl  2 0.56996 0.28498  3.1316 0.061879 . 
Residuals    24 2.18400 0.09100                    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

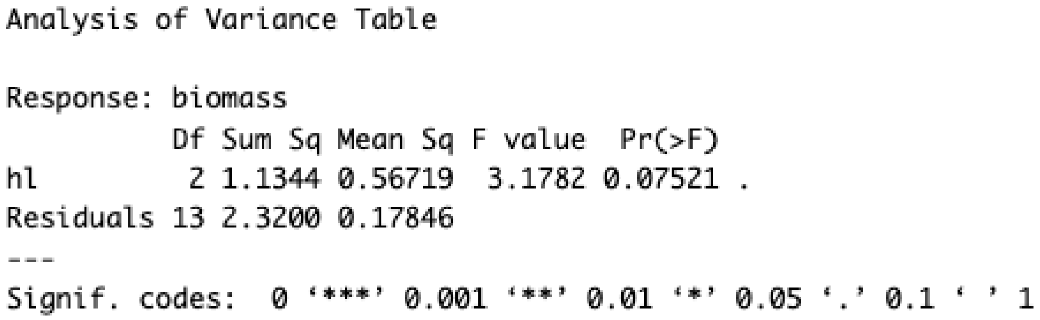


```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = biomass ~ treatment * hl, data = biomass.hf)

$treatment
          diff         lwr       upr     p adj
CT-BSA 0.17984 -0.15706245 0.5167425 0.3912252
NH-BSA 0.37000  0.03309755 0.7069025 0.0294326●
NH-CT  0.19016 -0.14674245 0.5270625 0.3519771

$hl
          diff       lwr        upr     p adj
L-H -0.3993067 -0.626648 -0.1719653 0.0013506●

$`treatment:hl`
                diff         lwr         upr     p adj
CT:H-BSA:H  -0.07120 -0.66110285  0.51870285 0.9989285
NH:H-BSA:H   0.44000 -0.14990285  1.02990285 0.2302206
BSA:L-BSA:H -0.52000 -1.10990285  0.06990285 0.1066004
CT:L-BSA:H  -0.08912 -0.67902285  0.50078285 0.9968739
NH:L-BSA:H  -0.22000 -0.80990285  0.36990285 0.8540171
NH:H-CT:H    0.51120 -0.07870285  1.10110285 0.1166710
BSA:L-CT:H  -0.44880 -1.03870285  0.14110285 0.2128044
CT:L-CT:H   -0.01792 -0.60782285  0.57198285 0.9999988
NH:L-CT:H   -0.14880 -0.73870285  0.44110285 0.9683400
BSA:L-NH:H  -0.96000 -1.54990285 -0.37009715 0.0004926 ●
CT:L-NH:H   -0.52912 -1.11902285  0.06078285 0.0969546 ●
NH:L-NH:H   -0.66000 -1.24990285 -0.07009715 0.0220964 ●
CT:L-BSA:L   0.43088 -0.15902285  1.02078285 0.2493396
NH:L-BSA:L   0.30000 -0.28990285  0.88990285 0.6233296
NH:L-CT:L   -0.13088 -0.72078285  0.45902285 0.9818355
```

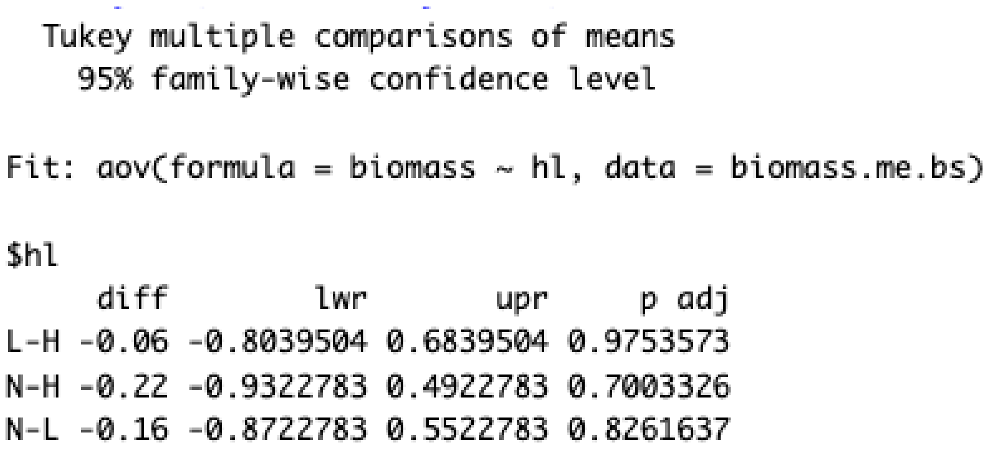
Biomass Mycena NH high/low and No N



```
Call:
lm(formula = biomass ~ hl, data = biomass.me.bs)

Residuals:
   Min     1Q Median     3Q    Max 
-0.600 -0.260 -0.070  0.155  0.800 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)   
(Intercept)   0.8200     0.1992   4.116  0.00122 **
hlL          -0.0600     0.2818  -0.213  0.83467   
hlN          -0.2200     0.2698  -0.816  0.42945   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4455 on 13 degrees of freedom
Multiple R-squared:  0.05299,	Adjusted R-squared:  -0.0927 
F-statistic: 0.3637 on 2 and 13 DF,  p-value: 0.7019
```

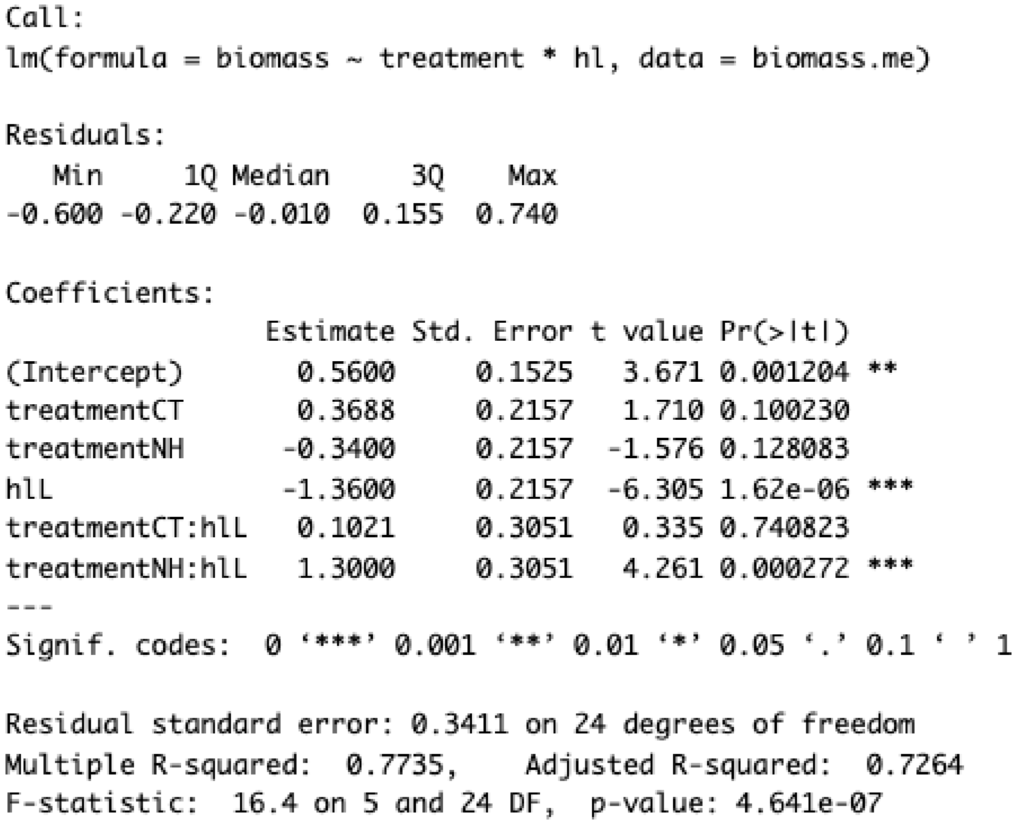


```
Analysis of Variance Table

Response: biomass
          Df Sum Sq Mean Sq F value  Pr(>F)  
hl         2 1.1344 0.56719  3.1782 0.07521 .
Residuals 13 2.3200 0.17846                  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

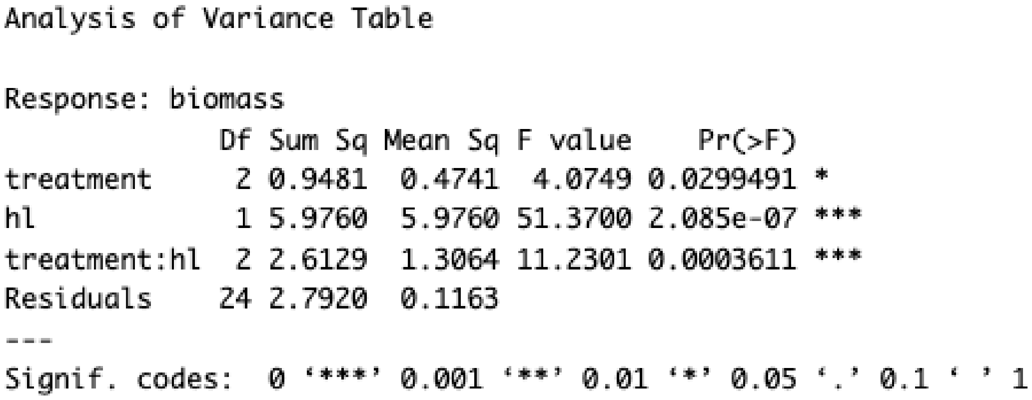


```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = biomass ~ hl, data = biomass.me.bs)

$hl
     diff        lwr       upr     p adj
L-H -0.06 -0.8039504 0.6839504 0.9753573
N-H -0.22 -0.9322783 0.4922783 0.7003326
N-L -0.16 -0.8722783 0.5522783 0.8261637
```

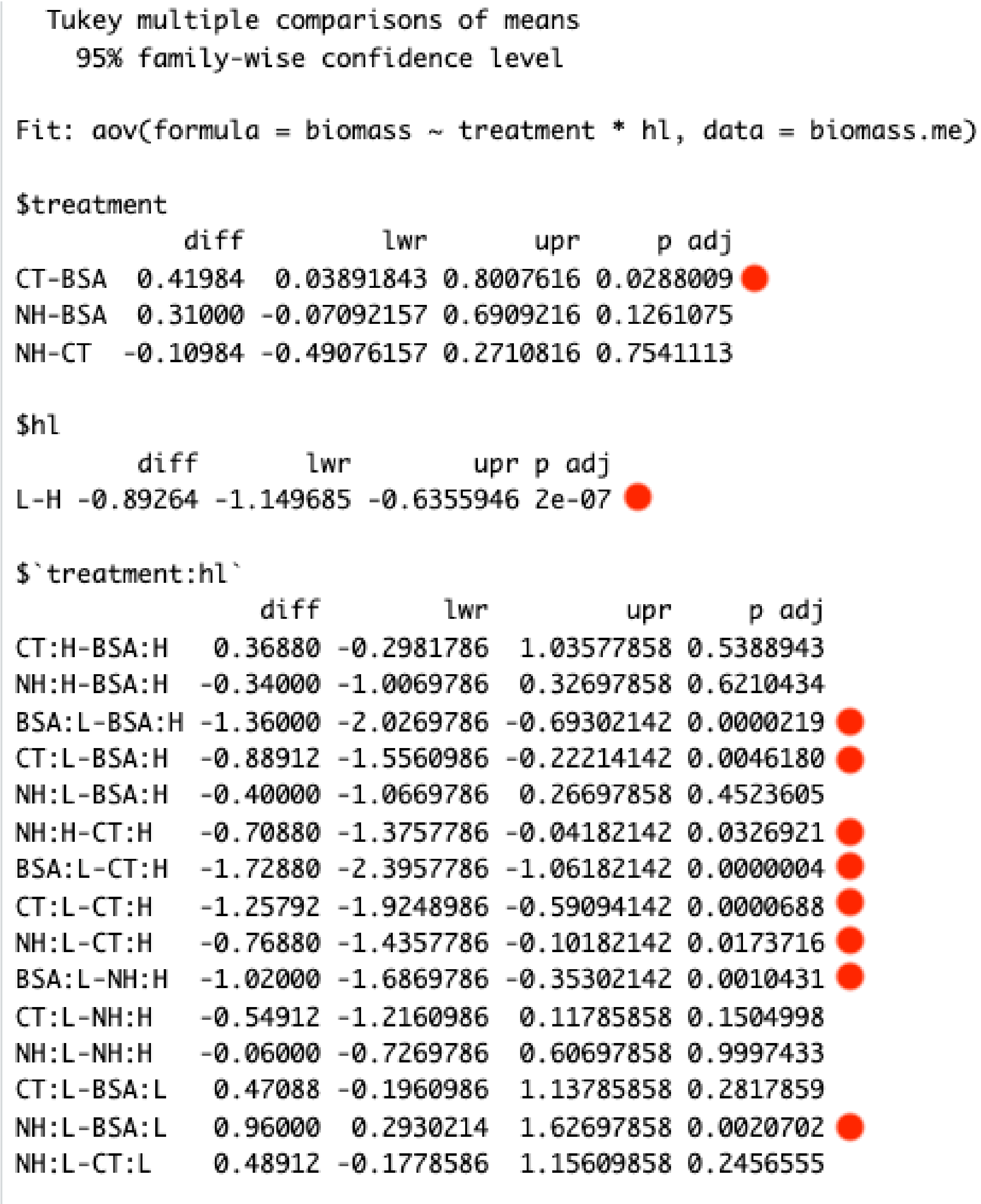
Biomass Mycena



```
Call:
lm(formula = biomass ~ treatment * hl, data = biomass.me)

Residuals:
   Min     1Q Median     3Q    Max 
-0.600 -0.220 -0.010  0.155  0.740 

Coefficients:
                Estimate Std. Error t value Pr(>|t|)    
(Intercept)       0.5600     0.1525   3.671 0.001204 ** 
treatmentCT       0.3688     0.2157   1.710 0.100230    
treatmentNH      -0.3400     0.2157  -1.576 0.128083    
hlL              -1.3600     0.2157  -6.305 1.62e-06 ***
treatmentCT:hlL   0.1021     0.3051   0.335 0.740823    
treatmentNH:hlL   1.3000     0.3051   4.261 0.000272 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3411 on 24 degrees of freedom
Multiple R-squared:  0.7735,	Adjusted R-squared:  0.7264 
F-statistic:  16.4 on 5 and 24 DF,  p-value: 4.641e-07
```

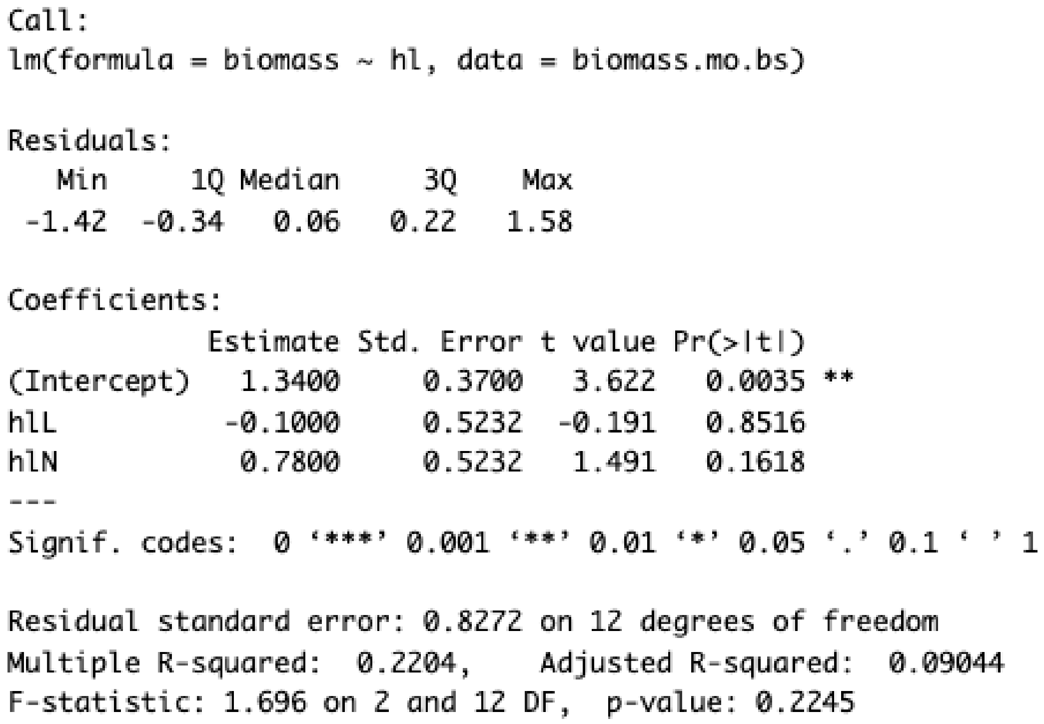


```
Analysis of Variance Table

Response: biomass
             Df Sum Sq Mean Sq F value    Pr(>F)    
treatment     2 0.9481  0.4741  4.0749 0.0299491 *  
hl            1 5.9760  5.9760 51.3700 2.085e-07 ***
treatment:hl  2 2.6129  1.3064 11.2301 0.0003611 ***
Residuals    24 2.7920  0.1163                      
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

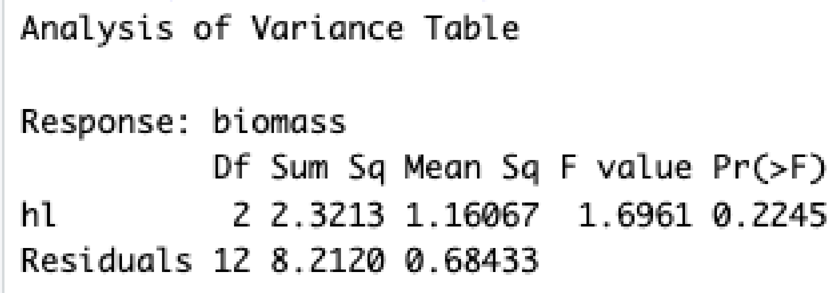


```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = biomass ~ treatment * hl, data = biomass.me)

$treatment
           diff         lwr       upr     p adj
CT-BSA  0.41984  0.03891843 0.8007616 0.0288009 ●
NH-BSA  0.31000 -0.07092157 0.6909216 0.1261075
NH-CT  -0.10984 -0.49076157 0.2710816 0.7541113

$hl
        diff       lwr        upr p adj
L-H -0.89264 -1.149685 -0.6355946 2e-07 ●

$`treatment:hl`
                diff        lwr         upr     p adj
CT:H-BSA:H   0.36880 -0.2981786  1.03577858 0.5388943
NH:H-BSA:H  -0.34000 -1.0069786  0.32697858 0.6210434
BSA:L-BSA:H -1.36000 -2.0269786 -0.69302142 0.0000219 ●
CT:L-BSA:H  -0.88912 -1.5560986 -0.22214142 0.0046180 ●
NH:L-BSA:H  -0.40000 -1.0669786  0.26697858 0.4523605
NH:H-CT:H   -0.70880 -1.3757786 -0.04182142 0.0326921 ●
BSA:L-CT:H  -1.72880 -2.3957786 -1.06182142 0.0000004 ●
CT:L-CT:H   -1.25792 -1.9248986 -0.59094142 0.0000688 ●
NH:L-CT:H   -0.76880 -1.4357786 -0.10182142 0.0173716 ●
BSA:L-NH:H  -1.02000 -1.6869786 -0.35302142 0.0010431 ●
CT:L-NH:H   -0.54912 -1.2160986  0.11785858 0.1504998
NH:L-NH:H   -0.06000 -0.7269786  0.60697858 0.9997433
CT:L-BSA:L   0.47088 -0.1960986  1.13785858 0.2817859
NH:L-BSA:L   0.96000  0.2930214  1.62697858 0.0020702 ●
NH:L-CT:L    0.48912 -0.1778586  1.15609858 0.2456555
```

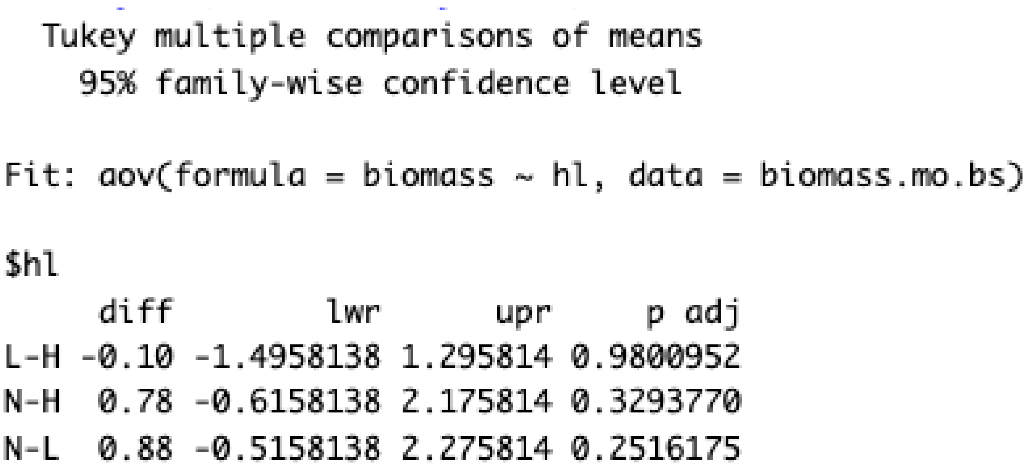
Biomass Marasmius NH high/low and No N



```
Call:
lm(formula = biomass ~ hl, data = biomass.mo.bs)

Residuals:
   Min     1Q Median     3Q    Max 
 -1.42  -0.34   0.06   0.22   1.58 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)   
(Intercept)   1.3400     0.3700   3.622   0.0035 **
hlL          -0.1000     0.5232  -0.191   0.8516   
hlN           0.7800     0.5232   1.491   0.1618   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.8272 on 12 degrees of freedom
Multiple R-squared:  0.2204,	Adjusted R-squared:  0.09044 
F-statistic: 1.696 on 2 and 12 DF,  p-value: 0.2245
```

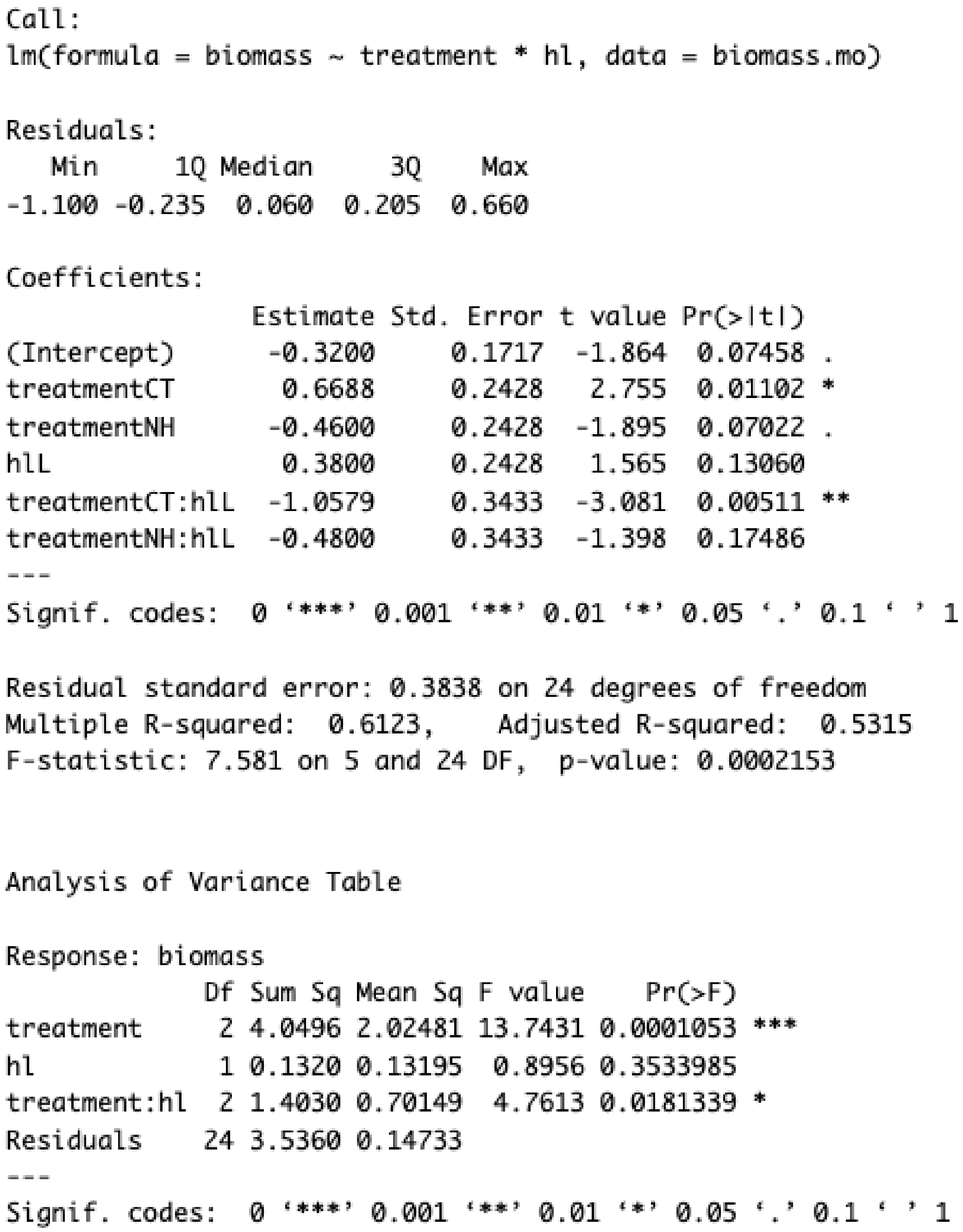


```
Analysis of Variance Table

Response: biomass
          Df Sum Sq Mean Sq F value Pr(>F)
hl         2 2.3213 1.16067  1.6961 0.2245
Residuals 12 8.2120 0.68433
```

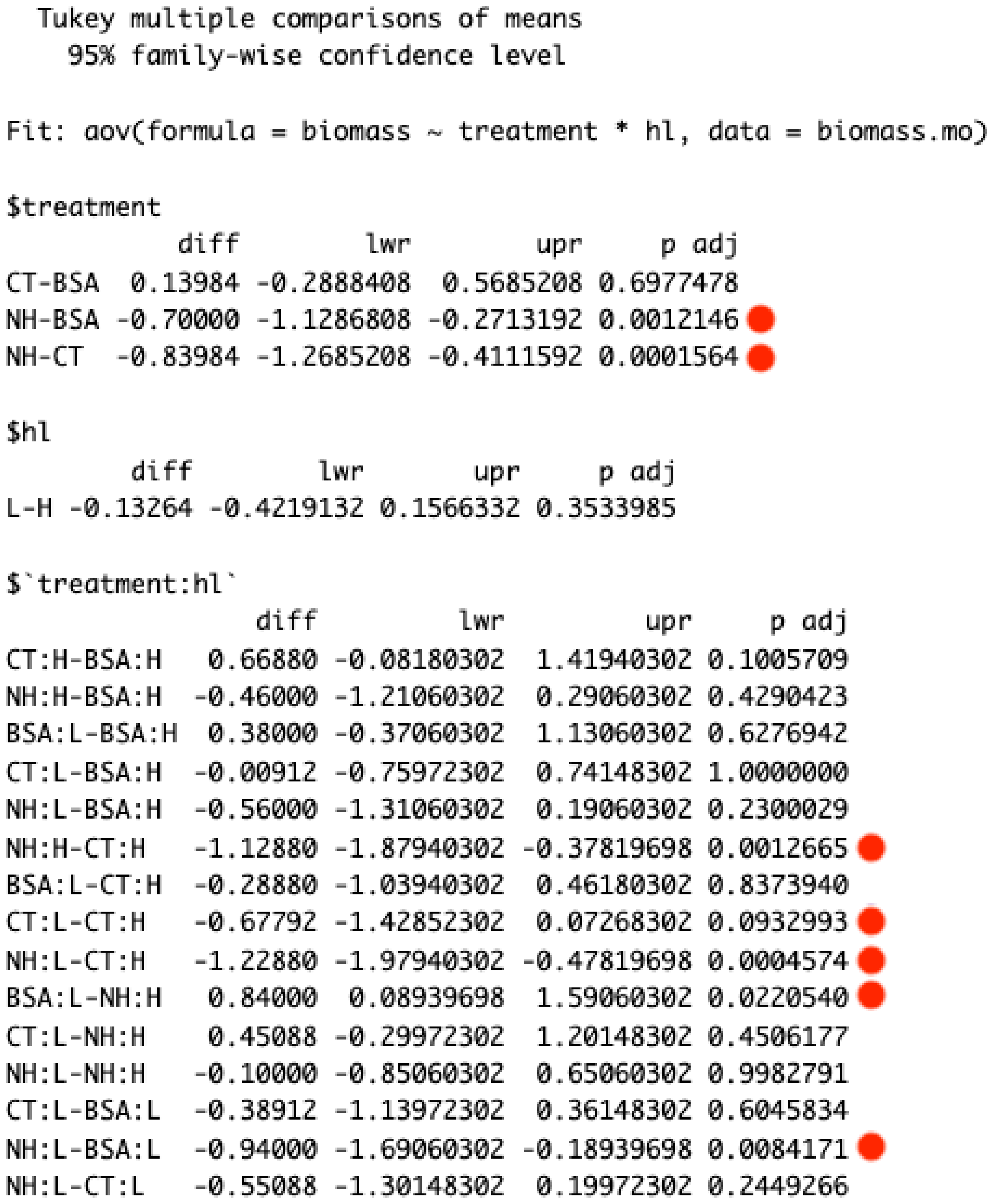


```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = biomass ~ hl, data = biomass.mo.bs)

$hl
     diff        lwr      upr     p adj
L-H -0.10 -1.4958138 1.295814 0.9800952
N-H  0.78 -0.6158138 2.175814 0.3293770
N-L  0.88 -0.5158138 2.275814 0.2516175
```

Biomass Marasmius



```
Call:
lm(formula = biomass ~ treatment * hl, data = biomass.mo)

Residuals:
   Min     1Q Median     3Q    Max 
-1.100 -0.235  0.060  0.205  0.660 

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)   
(Intercept)       -0.3200     0.1717  -1.864  0.07458 . 
treatmentCT        0.6688     0.2428   2.755  0.01102 * 
treatmentNH       -0.4600     0.2428  -1.895  0.07022 . 
hlL                0.3800     0.2428   1.565  0.13060   
treatmentCT:hlL  -1.0579     0.3433  -3.081  0.00511 **
treatmentNH:hlL  -0.4800     0.3433  -1.398  0.17486   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3838 on 24 degrees of freedom
Multiple R-squared:  0.6123,	Adjusted R-squared:  0.5315 
F-statistic: 7.581 on 5 and 24 DF,  p-value: 0.0002153


Analysis of Variance Table

Response: biomass
             Df Sum Sq Mean Sq F value    Pr(>F)    
treatment     2 4.0496 2.02481 13.7431 0.0001053 ***
hl            1 0.1320 0.13195  0.8956 0.3533985    
treatment:hl  2 1.4030 0.70149  4.7613 0.0181339 *  
Residuals    24 3.5360 0.14733                      
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = biomass ~ treatment * hl, data = biomass.mo)

$treatment
           diff        lwr        upr     p adj
CT-BSA  0.13984 -0.2888408  0.5685208 0.6977478
NH-BSA -0.70000 -1.1286808 -0.2713192 0.0012146 ●
NH-CT  -0.83984 -1.2685208 -0.4111592 0.0001564 ●

$hl
        diff        lwr       upr     p adj
L-H -0.13264 -0.4219132 0.1566332 0.3533985

$`treatment:hl`
                diff         lwr         upr     p adj
CT:H-BSA:H   0.66880 -0.08180302  1.41940302 0.1005709
NH:H-BSA:H  -0.46000 -1.21060302  0.29060302 0.4290423
BSA:L-BSA:H  0.38000 -0.37060302  1.13060302 0.6276942
CT:L-BSA:H  -0.00912 -0.75972302  0.74148302 1.0000000
NH:L-BSA:H  -0.56000 -1.31060302  0.19060302 0.2300029
NH:H-CT:H   -1.12880 -1.87940302 -0.37819698 0.0012665 ●
BSA:L-CT:H  -0.28880 -1.03940302  0.46180302 0.8373940
CT:L-CT:H   -0.67792 -1.42852302  0.07268302 0.0932993 ●
NH:L-CT:H   -1.22880 -1.97940302 -0.47819698 0.0004574 ●
BSA:L-NH:H   0.84000  0.08939698  1.59060302 0.0220540 ●
CT:L-NH:H    0.45088 -0.29972302  1.20148302 0.4506177
NH:L-NH:H   -0.10000 -0.85060302  0.65060302 0.9982791
CT:L-BSA:L  -0.38912 -1.13972302  0.36148302 0.6045834
NH:L-BSA:L  -0.94000 -1.69060302 -0.18939698 0.0084171 ●
NH:L-CT:L   -0.55088 -1.30148302  0.19972302 0.2449266
```

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