# Mechanisms of pH change
# in wood ant (*Formica polyctena*) nests

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## Master thesis

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*Annotation:* The aim of this study was to reveal mechanisms of pH change in wood ant nests. Contents of basic cations and glucose brought in ant food, i.e., honeydew and prey, into the nests were measured. Manipulation experiment was carried out to test effects of glucose and $Ca^{2+}$ cations brought by ants into the nest on increase in pH.

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# Table of Contents

# 1. Introduction

Wood ants (*Formica* s.str.) are known as ecosystem engineers influencing many parts of temperate and boreal forest ecosystems (Dlusskij 1967; Frouz and Jílková 2008). They affect energy and nutrient flow, food web structure, as well as soil properties due to their activities. They build large and long-lived nests that may comprise up to several millions individuals (Seifert 1996). Although materials for construction of the nest come from the nest surroundings, properties of the nest material differ in comparison to the surrounding forest floor (Dlusskij 1967; Frouz and Jílková 2008; Jílková et al. 2011). One of the properties that differ is pH and mechanisms of this change are not yet completely understood.

Ants bring large amounts of food, i.e., honeydew and prey, and plant materials into their nests (Horstmann 1974; Pokarzhevskij 1981). Glucose, which is found in a large proportion in honeydew, is considered to be a priming effect for soil microorganisms in the process of decomposition (Joergensen and Scheu 1999; Seeger and Filser 2008; Stadler et al. 1998). Nutrients, such as basic cations, are released from organic materials during the process of decomposition (Frouz et al. 1997, 2003). Basic cations are known to increase pH as they act in the cation exchange complex (Brady and Weil 2002). Through these effects pH of the ant nest material could be changed.

The aim of this study is to contribute to the revelation of mechanisms that lead to the change in pH. Especial interest was given to:

1. the determination of contents of glucose and basic cations that are brought by ants to the nest

2. the effects of glucose and basic cations on pH change in the ant nest material.

# 2. Review

## 2.1 Biology of wood ants

### 2.1.1 Area of distribution

Wood ants (*Formica* s.str., Hymenoptera, Formicidae) include dominant species of ants in temperate and boreal forest ecosystems (Dlusskij 1967). The group of wood ants comprises these species: *Formica rufa* Linnaeus 1758, *Formica polyctena* Förster 1850, *Formica lugubris* Zetterstedt 1838, *Formica aquilonia* Yarrow 1955, *Formica truncorum* Fabricius 1804 and *Formica pratensis* Retzius 1783 (Seifert 1996). Wood ants are widespread from the temperate zone of Europe and Asia to the North-Palaearctic area, even north of the Arctic Circle (Czechowski et al. 2002; Punttila and Kilpeläinen 2009; Seifert 1996). Wood ant species are mainly associated with coniferous and mixed forests, though they also occur in deciduous ones (Czechowski et al. 2002). They occupy sunny places, forest clearings and edges, but they are also found in shaded places.

### 2.1.2 Ant colonies

Wood ant colonies are monogynous (one queen and up to several hundred thousand workers; usually *Formica rufa*) or more often polygynous (up to a few thousand queens and one million workers; usually *Formica polyctena*) (Czechowski et al. 2002; Seifert 1996). New colonies are initiated by one queen alone (monogynous form), but mainly by colony fission (polygynous form). The second type gives arise to a polycalic system that often comprises a high number of nests that cover large extents of forest (Czechowski et al. 2002; Zacharov et al. 1981).

## 2.1.3 Food of wood ants

Wood ants are omnivorous; they forage for invertebrate prey as well as for honeydew from aphids, plant materials and sap (Horstmann 1974; Petal 1978; Stradling 1978). *Formica spp.* is feeding largely on honeydew rather than on solid insect prey; honeydew rarely forms less than two-thirds of the energy returned to the nest of *Formica rufa* (Whittaker 1991). Protein-rich insect prey is consumed mainly by brood, whereas sugar-rich honeydew is utilized by workers (Sorensen and Vinson 1981). The composition of the diet of an ant colony may be a reflection of its own specialization or the relative availability of different food materials in its environment (Stradling 1978). The crop volume of foraging ants reaches a maximum in spring and late summer, which probably depends on the abundance of the main producer of honeydew, *Lachnus roboris* L. (Horstmann 1972). The amount of prey brought into the nest attains a maximum in May, which coincides with the occurrence of the moth *Tortrix viridana* (Horstmann 1972), or in July and August, which coincides with the occurrence of leafhoppers (Typhlocybinae) (Whittaker 1991).

Species of aphids that wood ants most commonly tend are *Lachnus roboris* in the oak (Horstmann 1972), *Cinara spp.* feeding exclusively on Norway spruce (*Picea abies*) (Stadler et al. 1998), or *Symydobius oblongus*, which lives in the birch (Laine and Niemelä 1989). Prey collected by wood ants comprises predominantly Diptera (Sciaridae, Culicidae and Brachycera), Coleoptera (Curculionidae and Staphylinidae), Lepidoptera (Tortricidae), Hemiptera (Aphidina), Myriapoda (Diplopoda), Arachnoida (Araneida and Opiliones) and Oligochaeta (Domisch et al. 2009; Horstmann 1972, 1974; Stradling 1978).

Ants forage for food in the ant nest surroundings. The size of a foraging area depends on the ant species, the size of a population and workers, the availability of food in the area, terrain characteristics, and also on the presence of territories of adjacent nests (Petal 1978; Whittaker 1991), e.g., the length of foraging trails of *Formica rufa* recorded by Whittaker

(1991) was 60 m, whereas Stradling (1978) recorded the length of more than 100 m. The number of foraging trails spreading radially from the mound is six on average and they pass near aphid-bearing trees (Laine and Niemelä 1989). The intensity of ant foraging is obviously related to the distance from the nest (Whittaker 1991). The fall-off in the flow rate on *Formica rufa* ant trails is exponential and usually reaches zero at about 60 m from the nest. Wood ants are active from April to early November (Horstmann 1972), the activity is highest during the summer months and generally increases linearly with temperature (Domisch et al. 2009).

The workers of a medium-sized nest of *Formica polyctena* ants, which occupy a territory of 0.27 ha, collect on average 6.1 million prey pieces (a volume of 28 l, a nitrogen content of 280 g and an energy content of $6.2 \times 10^4$ kcal) and 155 l honeydew (a sugar content of 41 kg, a nitrogen content of 100 g and an energy content of $16.4 \times 10^4$ kcal) (Horstmann 1974). Food is accumulated in the nest in larger quantities than a colony requires, and so the storage of food makes ants independent of the actual food supply in the habitat and enables them to stay within the nest during unfavourable daily and seasonal changes in temperature and humidity (Petal 1978). Huge amounts of organic materials are concentrated in ant nests due to foraging activities of ants and a portion of these materials is utilized by soil microorganisms in the process of decomposition (Frouz et al. 1997, 2003).

### 2.1.4 Wood ant nests

Wood ants build large and long-lived mound nests that can be occupied even for decades (Hölldobler and Wilson 1990; Seifert 1996). An ant nest consists of belowground chambers, a soil rim and a mound of a conical shape (Dlusskij 1967; Frouz et al. 2005). Belowground chambers are digged in the mineral soil and can reach more than 50 cm in depth (*Formica polyctena*) (Kristiansen and Amelung 2001). The rim on the periphery is composed of fine

organic material and soil excavated by burrowing chambers and corridors in the belowground part of the nest. Organic debris and excrements of ants are usually deposited here. The rim can reach several meters in diameter and up to 40 cm in height. The organic mound of a conical shape is built of needles, small branches and other plant material collected from the nest surroundings and salivary secretion of ants used as a cementing substance (Petal 1978). This part of an ant nest can be taller than 1 m. Annually the input of plant remains (needle litter) to the nest constitutes 12-37% of the plant nest material (350 kg per ha) (Pokarzhevskij 1981). The surface layer usually consists of finer plant material, whereas the deep layers comprise larger pieces of plant material. The mounds are often constructed around stumps, which offer suitable microclimate at the beginning of nest construction and in early spring when temperatures are low (Gösswald 1938; Laine and Niemelä 1989).

## 2.2 Microclimate in wood ant nests

Wood ants can regulate microclimatic conditions in their nests by their activities. These conditions appropriate more or less the optimum of ants and are independent of climatic conditions on latitudinal or altitudinal gradient (Petal 1980; Seifert 1996). Although the temperature optimum of wood ants ranges from 22 to 32°C, their nests with maintained microclimate allow them to live in cold regions and thus wood ants are widespread mainly in the boreal zone of the northern hemisphere (Seifert 1996).

### 2.2.1 Moisture

Many authors have found lower water content in ant nests in comparison to the nest surroundings (e.g., Laakso and Setälä 1997; Lafleur et al. 2002). Although there are several sources of water in the nests, including condensation of air water, rain, fog and metabolic water produced by ants (Frouz 2000), several mechanisms exist that allow nest material to

stay dry. A conical shape of the mound and its maintained surface layer prevent nests from infiltration of rain water (Seifert 1996). Belowground parts of the nest are porous, which allows water to run quickly through the nest and improves aeration and evaporation of water from the nest material (Lafleur et al. 2002; Petal 1978).

The moisture of the nest material is higher during the period of ant activity (Frouz 1996). In occupied nests, the highest water content can be found in the top part of the nest. Moisture of nest material is comparatively stable at least in the period of ant activity. The established differences between the occupied and abandoned nests as well as the maintenance of stable water content during the period of ant activity suggest that the nest moisture can be controlled by ants.

Water content correlates with nest volume and degree of shading (Frouz 1996; Frouz 2000). As a result, wood ant nests are divided into two groups according to their water content. Dry nests have less than 20% moisture and wet nests have more than 35% moisture. Dry nests are more frequent than wet ones; they are often smaller and occur on sunny places where they are well exposed to sun. Wet nests are usually bigger and are situated in shaded places.


## 2.2.2 Temperature

The temperature in ant mounds is maintained during about eight months (April-November), which responses to the period of ant activity (Rosengren et al. 1987). But even in winter there were recorded temperatures above 1-2°C in ant mounds. Ants maintain daily average temperature higher than 20°C in their nest for a relatively short period of the year (65-129 days) (Frouz and Finer 2007). Annual nest temperatures peak in June; minimum temperature is reached from February to March (Frouz and Finer 2007). Summer temperatures are on average about 7-12°C higher than air temperatures. The hottest point in

the nest is located at a depth of 50 cm from the top; the centre of the ant nest is twice as warm as the surface layer (Coenen-Staβ 1980).

There are several sources of heat in ant nests. Solar radiation can warm up the surface of the ant mound (Seifert 1996) and can also accumulate in the bodies of workers active outside the nest (Frouz 2000). Since workers are dark and have high water content, they have relatively high thermal capacity. The thermal energy accumulated in the workers' bodies raise the temperature inside the nest. Ant workers producing metabolic heat cluster together in the centre of the nest (Frouz 2000). Ant metabolic heat production may be regulated by ant aggregation and dispersion. Intranest temperatures reach a high level in spring because ants collect fresh material and reorganize nest architecture after the winter, thus creating favourable conditions for heat-generating aerobic microorganisms (Rosengren et al. 1987). The heat production of nest material depends on several conditions such as temperature, humidity, composition of nest material, and oxygen concentration. The mass-specific heat production of ants is clearly higher than that of nest material. But considering the total mass of the nest, the total heat production of nest material is more than seven times the heat produced by ants.

The temperature in the nest of wood ants depends on climatic conditions (solar radiation, temperature, wind, and humidity of the air and soil), as well as on the ant population density, the size of a mound, nest moisture and shading (Coenen-Staβ 1980; Frouz 2000; Frouz and Finer 2007; Petal 1978).

The moisture of nest material was found to be the main factor affecting thermal properties of nests (Frouz 1996). Thus, wet and dry nests differ in the thermal regime and capacity (Frouz 1996; Frouz 2000; Frouz and Finer 2007). The temperature of the surface layer is higher in wet nests. On the other hand, the thermal loss is about 15 times higher in wet nests than in dry ones because of the higher thermal capacity of wet nests. The thermal losses are

compensated by higher respiration of ants and higher microbial heat production as microbial heat production is closely related to nest moisture.

## 2.3 Ants' influence on soil properties

Ants are considered as ecosystem engineers due to their effects on energy flow, nutrient transportation, food web structure and soil properties (Dlusskij 1967; Frouz and Jílková 2008; Jones et al. 1994; Jouquet et al. 2006). Soil engineering activities of ants are shown in Figure 1. Mound building ants collect material for construction of their nests and food for their colonies in the nest surroundings. They also provide soil engineering activities due to the construction of nests. Favourable microclimate is maintained in ant nests, which provides optimum conditions for microbial activity. Thus they are influencing many parts of ecosystems, especially soil physical, chemical, and biological properties. Differences in nutrient contents and micro flora abundance between species are probably caused by differences in their ways of life, feeding and nest construction (Petal 1978).
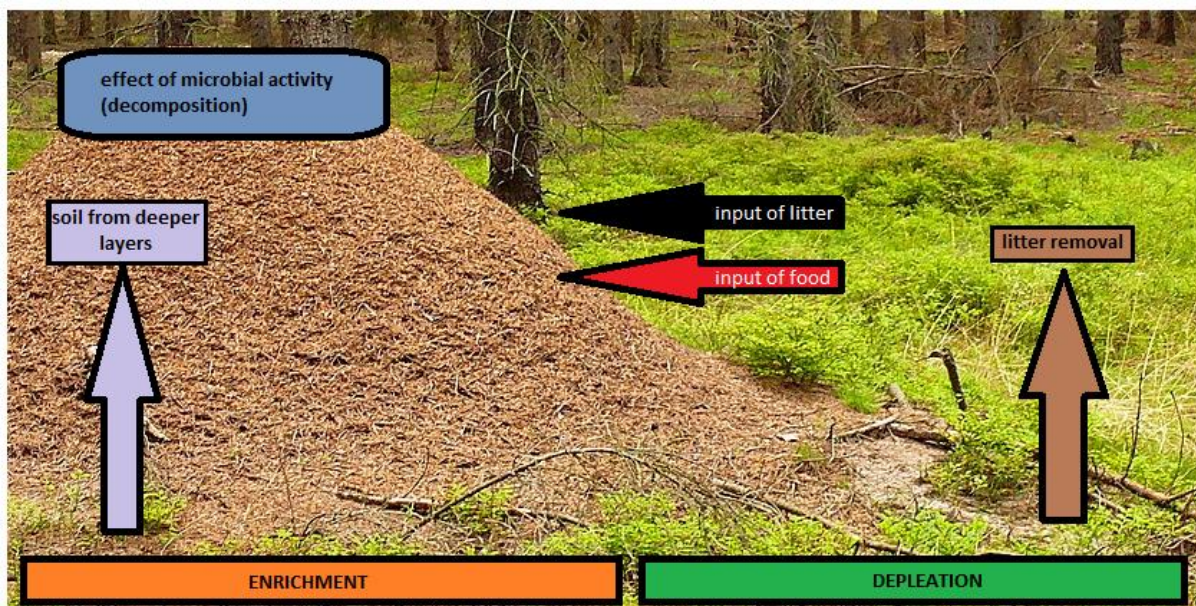


**Figure 1:** Soil engineering activies of ants in forest ecosystems.

## 2.3.1 Physical properties

Building of tunnels and chambers and mixing activities of ants increase soil porosity and reduce bulk density (Frouz and Jílková 2008; Kilpeläinen et al. 2007; Kristiansen and Amelung 2001). Reduced bulk density may increase soil aeration and permeability of soil for water (Dlusskij 1967; Frouz and Jílková 2008). Smaller particles are transported from deeper layers to the surface, whereas soil organic matter is incorporated into the belowground parts of the nest (Petal 1978).

## 2.3.2. Chemical properties

Wood ants change chemical properties of nests and their surroundings due to mixing of soil and transporting of organic materials and food into ant nests (Frouz and Jílková 2008; Jílková et al. 2011). Nest chemical properties are mostly dependent on the quality of litter used for nest construction, whereas the properties of the forest floor are dependent on both, litter and parent material (Kilpeläinen et al. 2007; Lenoir et al. 2001).

Ant nests are enriched with organic matter and mineral products of its decomposition – nitrogen and its inorganic compounds ($NO_3^-$ and $NH_4^+$), carbon, phosphorus and basic cations ($Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$) in comparison to the surrounding soil (Frouz et al. 1997; Laakso and Setälä 1997; Lenoir et al. 2001). Differences between ant nests and the surrounding soil are most pronounced in the upper parts of the nests (Frouz et al. 2003).

One of the soil properties influenced by ants is pH. Soil pH is shifted towards neutral values, i.e., nests have higher pH than surrounding acidic soil and lower pH than surrounding alkaline soil (Dlusskij 1967; Frouz and Jílková 2008). Soil pH of ant nests and their surroundings depends on the soil type (Petal 1978). The increase is most probably caused by the accumulation of exchangeable cations, whereas the decrease is caused by enhanced

amounts of organic matter, especially in the nest surroundings (Jílková et al. 2011; Petal 1978).

Soil chemical properties are changed by ant activities even in wider surroundings of the nest, cca up to 8 m diameter (Jílková et al. 2011). The area influenced by ants varies with the size of ant colony and also with the ant species (Petal et al. 2003; Wagner et al. 2004).

## 2.3.3 Biological properties

The functioning of most terrestrial ecosystems is typically influenced by the availability of nutrients and water (Duvigneaud and Denaeyer-De Smet 1970). Wood ants due to their activities improve microclimatic conditions and availability of nutrients in their nests and thus influence plants growing in the nest surroundings and soil fauna and microorganisms living in the nests and their surroundings.

### 2.3.3.1 Plants

There are enhanced both nutrient concentrations and nutrient availability to plants in ant nests (Frouz et al. 2005; Lafleur et al. 2002). Higher availability of nutrients is caused especially by higher pH in ant nests (Frouz et al. 2003). Mineral forms of nitrogen and phosphorus are considered to be limiting to plant growth in coniferous forests (Lafleur et al. 2002, 2005). Contents of both nutrients are enhanced in ant mounds, which together with favourable pH encourage plant growth in the nest surroundings (Dlusskij 1967; Zacharov et al. 1981).

Fine root biomass and biomass density are higher in the belowground parts of nests than in the surrounding mineral soil (Ohashi et al. 2007). Wood ant mounds increase heterogeneity in root distribution in forest ecosystems and also increase the availability of nutrients for plants that extend their roots inside the mounds.

Plants are usually absent from the nest surface because of the dry conditions or because of the reconstruction activities of ants (Petal 1978). Ants can eliminate plants by biting their roots or by burying them in the soil (Woodel and King 1991). Not only the nest site but also the area of 1-2 m around the nest and foraging trails tend to be bare of plants (Crist and MacMahon 1991; MacMahon et al. 2000; Petal 1978; Woodel and King 1991). Plant species associated with ant nests usually differ from species growing in adjacent areas (Dean et al. 1997).

### 2.3.3.2 Soil fauna

Higher abundance and functional diversity have been found in ant nests in comparison to the adjacent soil. There are enhanced numbers of individuals of various groups of soil fauna: Nematoda, Tardigrada, Oligochaeta and Arthropoda (Acari, Collembola, Insecta) (Laakso and Setälä 1998). For example, a much more abundant earthworm community harbours the ant nest mound surface than the surrounding soil, with *Dendrobilus rubidus* dominating this community (Laakso and Setälä 1997). Favourable microclimate together with abundant food supply is likely to make a nest mound a preferred habitat for earthworms. Earthworms probably prevent the nest mounds from becoming overgrown by moulds and fungi, indicating possible mutuality relationship between the earthworms and the ants.

### 2.3.3.3 Soil microorganisms

Total microbial biomass is about three times higher in the nest material than in the soil (Laakso and Setälä 1997). The abundance of bacteria and fungi is usually enhanced in ant nests, whereas the abundance of actinomycetes is lower. Decomposer microbes are more active and abundant in the warm and moist surface layer (Frouz et al. 2003; Laakso and Setälä

1998). Soil microorganisms are responsible for decomposition of soil organic matter (Brady and Weil 2002; Lenoir et al. 2001).

## 2.4 Forest ecosystem processes

### 2.4.1 Ant-tended aphids and their effects on ecosystems

Many ant species have mutuality relationships with aphids feeding on the phloem sap of a wide variety of plants (Hölldobler and Wilson 1990). Wood ants protect aphids from predators, and in turn, are supplied with honeydew excreted by aphids. Ants may collect more than 80% of the excreted honeydew, which constitutes 62-94% of their diet and contains 15-20% sugars and a small amino acid component, the rest (about 70%) is made up of water (Rosengren and Sundström 1991).

Phloem sap consists mainly of a concentrated solution of simple sugars and a weak solution of amino acids (Dixon 2005). Aphids live in an environment that is nutritionally very favourable in spring and autumn but less so in summer. Aphids produce large quantities of honeydew, which in some cases contains a high percentage of the trisaccharide sugar melezitose. They do this mainly by converting mono- and disaccharides into trisaccharides in order to reduce the osmotic concentration of honeydew. Aphids are able to process large quantities per unit time and extract sufficient amino-nitrogen to fuel their very high rates of growth. Their symbionts play an important role in that process.

Annual honeydew production by *Cinara* aphids in pine woods can be as high as 400-700 kg fresh mass per ha (Stadler at al. 1998). A large portion of this honeydew reaches ground level, which can result in as much as 10 g of sugar per 100 g of soil (Dixon 2005). The addition to the soil of the four sugars (fructose, glucose, melezitose and sucrose) commonly found in honeydew, causes an increase in the abundance of bacteria in woodland soils (Dixon 2005; Seeger and Filser 2008).

Sap-sucking insects may cause enlarged growth (galls), foliage disturbances such as chlorosis and deformations, and disease transmission (Franklin 1970). Sap consumption deteriorates the physiological condition of the tree and reduces foliage and wood production, but not to the extent as does the consumption of photosynthetic tissues by phytophages. Significant losses of timber growth in the vicinity of ant nests in European coniferous forests have been documented, and this has been attributed to the increase of ant-tended aphid species (Whittaker 1991). The results of Kilpeläinen et al. (2009) suggest that ant-aphid mutualism can have a significant effect on the growth of individual trees, but the effect is negligible at the ecosystem level. Nutrient removal in honeydew collected by ant-tended aphids is slowing growth of tree (Frouz et al. 2008). However, trees may partly compensate for this depletion by having access to a larger supply of soil nutrients near ant nests. Wood ants clearly reduce the numbers of some defoliating tree herbivores, but on the other hand they actively defend tended aphids against their predators (Rosengren and Sundström 1991). As a result, the impact of wood ants and aphids on tree growth is very complex.

## 2.4.2 Decomposition processes in forests

Organic matter decomposition plays an important role in nutrient fluxes (Brady and Weil 2002; Edwards et al. 1970; Lenoir et al. 2001; Paul and Clark 1996; Steubing 1970). The destruction of organic substances depends upon the number, species and activity of soil micro flora and fauna. In temperate regions, litter appears fragmented mainly by earthworms, enchytraeid worms, diplopods, isopods, dipteran larvae, collembolans and oribatid mites. Fragmentation by the fauna accelerates microbial invasion. The soil includes five major groups of microorganisms – the bacteria, actinomycetes, algae, fungi and protozoa. Generally, the microorganisms are concentrated in the surface layers of the soil and their biomass declines with depth. Bacteria and fungi dominate in the soils; bacteria often predominate on

easily decomposed substrates while fungi continue to decompose complex organic materials. Especially in forest soils, the fungi play an important role in the decomposition of organic matter.

Water-soluble polyphenols, fulvic and humic acids and other substances are leached from decomposed litter into the soil – various anions and cations are released which percolate into deeper soil horizons. Forested soils in a leaching environment have predominantly fulvic acids.

Soil moisture, aeration, temperature, acidity, organic matter, C/N ratio, the relation between cellulose and lignin, and the presence of other carbohydrates influence decomposition processes. The major microbial groups find optimal conditions at 50-80% of the water holding capacity of the soil. High temperatures favour the decomposition processes. Most microorganisms are mesophiles with the highest activity in a range of 35-40°C. Bacteria and actinomycetes have optimal development in neutral or alkaline soils, while fungi over a wide pH range, and thus prevail in acidic soils. Soil microbes require a balance of nutrients to build their cells and extract energy, thus low C/N ratio favours decomposition. The addition of almost any energy-rich organic substance stimulates microbial growth and activity. Addition of simple sugars, especially glucose, in form of honeydew into the soil activates microorganisms and increases their metabolic activity (Joergensen and Scheu 1999; Seeger and Filser 2008; Stadler et al. 1998).

Wood ants maintain stable microclimate in their nests during the period of their activity and these favourable conditions are suitable for soil microorganisms and promote decomposition processes in ant nests (Frouz et al. 2003; Laakso and Setälä 1998). Microbial activity and decomposition processes are encouraged especially in moist surface layers of ant nests and in wet nests. Decomposition was found about six times higher in ant nests in comparison to the surrounding forest floor (Frouz et al. 1997). On the other hand, many

authors have recorded lower mass losses and thus lower rate of nutrient mineralization in the mounds than on the surrounding forest floor (e.g., Domisch et al. 2008; Kristiansen and Amelung 2001; Lenoir et al. 2001). These results can be attributed to the lower water content in their ant mounds as well as to different composition of mound material or nutrient concentrations of the organic matter (Domisch et al. 2008).

### 2.4.3 Soil pH in forests

One of the most important soil properties is pH. Acidity (or alkalinity) is an expression of the activity or concentration of $H^+$ ions present in a solution (Brady and Weil 2002; Šimek 2005). Active acidity is defined by the $H^+$ ion activity in the soil solution, whereas exchangeable acidity is associated with exchangeable $Al^{3+}$ and $H^+$ ions released by cation exchange (mainly for basic cations, i.e., $Ca^{2+}$, $Mg^{2+}$, $Na^+$ and $K^+$) from the adsorption complex of the soil.

The main contributor to soil acidity is the formation and subsequent dissociation of $H^+$ ions from carbonic acid ($H_2CO_3$), which is formed when Carbone dioxide gas dissolves in water. $CO_2$ comes from the respiration of roots and microorganisms as well as from the decomposition of organic matter. In addition to carbonic acid, many other organic acids, such as carboxylic and phenol acids in humic substances are also generated as microbes break down soil organic matter. The accumulation of organic matter itself tends to decrease soil pH. Organic matter forms soluble complexes with basic cations, thus facilitating the loss of these cations by leaching. Organic matter is a source of $H^+$ ions because it contains numerous acids from which these cations dissociate. Plant uptake of cations ($Ca^{2+}$, $Mg^{2+}$, $Na^+$ and $K^+$) and their exchange for $H^+$ ions also lead to acidification of soils.

Generally, forested soils are acidic, which is mainly caused by leaching of basic cations by percolating rain water and by high amounts of organic acids, such as fulvic and humic

acids. Forested soils are often situated on granitic parent material, which also contributes to acidic reaction of the soil (Lassoie and Hinckley 1991). These effects together give rise to the acid cation-unsaturated humus.

The degree of soil acidity or alkalinity affects many chemical and biological soil properties, e.g., compound solubility, cation saturation, availability of elements and activity of soil microorganisms. In strongly acid soils, the availability of the macronutrients (Ca, Mg, K, P, N and S) for root uptake is reduced, whereas availability of most micronutrient cations (Fe, Mn, Zn, Cu and Co) is increased, even to the extent of toxicity to higher plants and microorganisms. Most bacteria and actinomycetes most prosper at intermediate and high pH values. Fungi seem to function well over a wide pH range, and therefore tend to predominate in acid soils. Forest stands are tolerant to soil acidity. Optimum pH for coniferous trees ranges from 5 to 5.5 and for deciduous trees from 5.7 to 6.5.

# 3. Materials and Methods

## 3.1 Study site

The study was conducted in a 80 to 100-year-old forest dominated by Norway spruce (*Picea abies*) with sparse understory in the southern part of the Czech Republic, in the National Reserve Polánka (N 49°27'53", E 14°49'43") (Fig. 2), which was used in several previous studies (Frouz and Finer 2007; Frouz et al. 2003; Jílková et al. 2011). The forest contained several hundred nests of the wood ant *Formica polyctena* scattered over about 10-ha area. Average annual temperature at the site reaches 7.1°C and annual precipitation is about 586 mm (Frouz and Finer 2007).



**Figure 2:** Map of the National Reserve Polánka. The study site is restricted by a yellow square.

## 3.2 Input of basic cations into ant nests from food

### 3.2.1 Collection of honeydew and prey

Input of basic cations into ant nests was estimated in two main food sources, honeydew and prey. For this estimation, amount of honeydew and concentrations of basic cations in honeydew and prey were measured. A method of honeydew and prey collection as well as the number of workers returning to the nest per day and amount of prey brought to the nest was taken from Frouz et al. (1997).

Amount of basic cations imported in the honeydew into the nest was determined by analyzing the bodies of workers leaving and returning to the nest. The samplings of ants were made 3 times during the period of ant activity (30th June, 17th July and 10th September 2009) at 5 comparable nests. For the analyses, workers returning to the nest (at least 30 individuals in each sample) were collected on the trees surrounding the ant nests. The workers leaving the nest (at least 30 individuals in each sample) were collected on the trees at the beginning of each day's observations. The ants in each sample were immediately killed by ethyl-acetate fumes, dried and counted. Their bodies were then weighed and analyzed for basic cations.

To assess the amount of basic cations imported in prey, prey brought in mandibles of returning workers was sampled. The prey was hand sorted from workers which were sampled on foraging trails of 5 comparable nests. The prey was collected only once (17th July 2009) just to get a sample of prey of ants for determination of the amount of basic cations. The prey was then dried; weighed and basic cations' contents were measured.

### 3.2.2 Chemical analyses of basic cations

Ant bodies were mineralized using 2 ml $HNO_3$ and 0.2 ml $H_2O_2$ at 90-100°C. Samples were then diluted with deionized water in 10 ml savillex bottles and concentrations of basic

cations were quantified by atomic absorption spectrophotometer using SpectrAA 640 at 420,7 nm (Varian, Inc. USA).

### 3.2.3 Calculations of input of basic cations

Daily input of basic cations from honeydew into an individual nest ($bc_h$) was calculated using a formula:

$$bc_h = N_t \cdot (m_r \, bc_r - m_l \, bc_l) \; [g \; nest^{-1} \; day^{-1}]$$

In each sample of returning workers, the amounts of basic cations in the bodies were calculated as a multiple of dry mass of ant body ($m_r$) and contents of basic cations in the body ($bc_r$). The amounts of basic cations in the bodies of leaving workers were calculated in the same way (dry mass of ant body ($m_l$) and contents of basic cations in the body ($bc_l$)). The number of workers returning per day ($N_t$) is $279 \times 10^3$ on average (Frouz et al. 1997).

Daily input of basic cations from prey into the nest ($bc_p$) was calculated using a formula:

$$bc_p = W_p \cdot bc_m \; [g \; nest^{-1} \; day^{-1}]$$

where mean daily input of prey into one nest ($W_p$) is $254 \; g \; nest^{-1} \; day^{-1}$ on average (Frouz et al. 1997) and $bc_m$ means mean contents of basic cations.

Annual inputs of basic cations from honeydew and prey were calculated:

$$A \, bc_p \, (A \, bc_h) = bc_p \, (bc_h) \cdot 100 \; [g \; nest^{-1} \; year^{-1}]$$

as ants are active approximately 100 days in year (Frouz et al. 1997).

# 3.3 Effects of glucose and $Ca^{2+}$ cations on pH change

## 3.3.1 Manipulation experiment

Twelve mounds (volume of 0.02 $m^3$) were built from the litter from the forest floor to test effects of glucose and $Ca^{2+}$ cations on pH change in ant mounds. The mounds were built at least 200 m from the nearest ant mound to avoid ants' influence on the experiment. The area where wood ants forage reaches up to 150 m (Seifert 1996).

Ants bring 13-215 kg of dry mass of honeydew into one ant nest (volume of 1-3 $m^3$) per year (Frouz et al. 1997). Mounds in this study had volume of 0.02 $m^3$, which gives about 300 g of glucose per year, even if we take into consideration that about 90% is utilized by ants as a source of energy (Frouz et al. 1997) and that glucose represents a substantial part of honeydew (Dixon 2005). Dry mass of the litter used to build the mounds was about 5 kg. Ants are known to bring 12-37% of nest material into the nest annually (Pokarzhevskij 1981), which means in our case about 1 kg. 50% of this mass is decomposed in the nest and $Ca^{2+}$ cations in the litter are represented by 0.55% (Pedersen and Bille-Hansen 1999), which gives 2.5 g of $Ca^{2+}$ cations approximately. According to this study, ants are able to bring about 300 g of $Ca^{2+}$ cations to the nest of volume 1-3 $m^3$ per year, which gives about 6 g in our smaller mounds. In total, about 10 g of $Ca^{2+}$ cations can be brought to the nests of volume 0.02 $m^3$ annually. This means an addition of 30 g of $CaCO_3$.

The mounds were divided into four groups according to litter treatment. In the first group of three mounds, the litter was sprayed with 600 ml of deionized water, in the second group with 600 ml of 1:1 glucose:water solution, in the third group with 600 ml of deionized water with addition of 30 g of $CaCO_3$, and in the fourth group with 600 ml of 1:1 glucose:water solution with addition of 30 g of $CaCO_3$. The mounds were placed inside a fence to be prevented from disturbance by animals.

The litter bag technique was used to determine the changes in litter properties (Domisch et al. 2008). The needle litter was collected from the forest floor in a Norway spruce stand near České Budějovice. Litter from the forest floor was used, as the ants collect fallen litter material for constructing their mounds. About 60 g of air dried litter was put into polyester litter bags of size 15x20 cm and mesh size 1 mm (Fig. 3). Three litter bags were placed into each mound at the beginning of the experiment in May 2010 and were collected in October 2010. A total of 36 litter bags were used.



**Figure 3:** The litter bag.

## 3.3.2 Analyses of litter properties

The litter bags were transported to a laboratory. Dry mass, pH, contents of basic cations and quotient Q 4/6 were determined.

Dry mass of the litter was determined after drying at 105°C for 4 hours. pH was measured in a 1:10 litter:water suspension with a glass electrode. Basic cations were extracted in 1:8 litter:Mehlich-1 suspension. The concentrations were determined using ICP OES (iCAP 6500, Thermoscientific) under conditions recommended by manufacturer. The quality of the

analytical measurement was controlled using the standard reference materials BCR 701 (Lake sediment), NIST 2709 (San Joaquin soil), NIST 2710 (Montana I Soil) and NIST 1643 (Trace elements in water). Quotient Q 4/6 is a ratio of absorbancies of humus acids at 474 and 666 nm and corresponds in a reversed ratio to the degree of decomposition and polymerization of humus acids (Valla 2000). Humus acids were extracted for 12 hours in 0.2 N NaOH (1:20 litter:NaOH), the samples then were diluted with deionized water (1:1), and absorbance were measured using ELISA (Multi-Detection Microplate Reader, Synergy$^{TM}$ 2, BioTek) at 474 and 666 nm, and with NaOH diluted in deionized water (1:1) as a blank.

### 3.3.3 Statistical analyses

The data obtained from the manipulation experiment were analyzed using the program Statistica. General linear models with treatment and nest as categorical variables and nest as a random factor nested in treatment were used for determination of the influence of glucose and $Ca^{2+}$ cations on litter properties (dry weight, pH, contents of basic cations and quotient Q 4/6). Post-hoc test (Fisher LSD) was used to assess differences between individual treatments. Confidence intervals ($p<0.05$) were used for comparison of individual treatments with values of the original litter.

# 4. Results

## 4.1 Input of basic cations into ant nests from food

Mean dry mass of one ant leaving the nest was $235 \pm 20 \times 10^{-5}$ g and mean dry mass of one ant returning to the nest was $290 \pm 32 \times 10^{-5}$ g (Tab. 1). Mean dry mass of honeydew carried by one worker was $58 \pm 26 \times 10^{-5}$ g. Dry mass of honeydew carried into one nest per day was approximately 161 g nest$^{-1}$ day$^{-1}$, which gives about 16 kg per year.

Mean concentrations of basic cations in bodies of ants leaving and returning to the nest are shown in Table 1. The amounts of basic cations which one worker brought with honeydew were $0.47 \pm 0.24 \times 10^{-6}$ g ($Ca^{2+}$), $0.41 \pm 0.26 \times 10^{-6}$ g ($Mg^{2+}$), $3.5 \pm 1.78 \times 10^{-6}$ g ($K^{+}$) and $0.8 \pm 0.42 \times 10^{-6}$ g ($Na^{+}$). The amounts of basic cations imported with honeydew into one nest per one day were approximately 0.13 g nest$^{-1}$ day$^{-1}$ ($Ca^{2+}$), 0.11 g nest$^{-1}$ day$^{-1}$ ($Mg^{2+}$), 0.98 g nest$^{-1}$ day$^{-1}$ ($K^{+}$) and 0.22 g nest$^{-1}$ day$^{-1}$ ($Na^{+}$).

**Table 1:** Mean dry mass and mean concentrations of basic cations in one ant leaving and returning to the nest (mean ± SD).

|  | dry mass [g] | concentration [ug g$^{-1}$] | | | |
|---|---|---|---|---|---|
|  |  | $Ca^{2+}$ | $Mg^{2+}$ | $K^{+}$ | $Na^{+}$ |
| leaving | $235 \pm 20 \times 10^{-5}$ | 392 ± 254 | 1385 ± 67 | 8976 ± 246 | 2105 ± 171 |
| returning | $290 \pm 32 \times 10^{-5}$ | 464 ± 208 | 1256 ± 65 | 8428 ± 457 | 1951 ± 118 |

Mean concentrations of basic cations in prey were $10.2 \pm 18.9$ mg g$^{-1}$ ($Ca^{2+}$), $1.2 \pm 0.4$ mg g$^{-1}$ ($Mg^{2+}$), $5.6 \pm 2.2$ mg g$^{-1}$ ($K^{+}$) and $0.7 \pm 0.4$ mg g$^{-1}$ ($Na^{+}$). The amounts of basic cations

imported with prey into one nest per one day were approximately 2.59 g nest$^{-1}$ day$^{-1}$ (Ca$^{2+}$), 0.31 g nest$^{-1}$ day$^{-1}$ (Mg$^{2+}$), 1.42 g nest$^{-1}$ day$^{-1}$ (K$^+$) and 0.17 g nest$^{-1}$ day$^{-1}$ (Na$^+$).

The annual inputs of basic cations from food of *Formica polyctena* into one nest were 272 g Ca$^{2+}$ (259 g from prey and 13 g from honeydew), 42 g Mg$^{2+}$ (31 g from prey and 11 g from honeydew), 240 g K$^+$ (142 g from prey and 98 g from honeydew) and 39 g Na$^+$ (17 g from prey and 22 g from honeydew) (Table 2).

**Table 2:** Annual inputs of basic cations from food (prey and honeydew) into one *Formica polyctena* nest.

| | amount [g nest$^{-1}$ year$^{-1}$] | | | |
|---|---|---|---|---|
| | Ca$^{2+}$ | Mg$^{2+}$ | K$^+$ | Na$^+$ |
| prey | 259 | 31 | 142 | 17 |
| honeydew | 13 | 11 | 98 | 22 |
| **total** | **272** | **42** | **240** | **39** |

## 4.2 Effects of glucose and Ca$^{2+}$ cations on pH change

Both treatments, glucose (G) and CaCO$_3$ (Ca), had significant effects on pH change ($F_{(3,8)}$=7.93, p=0.009) (Fig. 4a). In G treatment, pH increase was significant in comparison to water treatment (W), but pH increased significantly more in Ca treatment, even in comparison to G treatment. Increase in pH in G-Ca treatment was also significant in comparison to G and W treatments. In G, Ca and G-Ca treatments, pH was enhanced, while in W treatment, pH decreased in comparison to the original litter.

Ca$^{2+}$ concentration was also significantly affected by glucose and CaCO$_3$ treatments ($F_{(3,8)}$=5.06, p=0.03) (Fig. 4b). Ca$^{2+}$ concentrations were not significantly different between W and G treatments as well as between W, Ca and G-Ca treatments, but were significantly increased in Ca and G-Ca treatments in comparison to G treatment. Ca$^{2+}$ concentrations in all treatments were enhanced in comparison to the original litter.

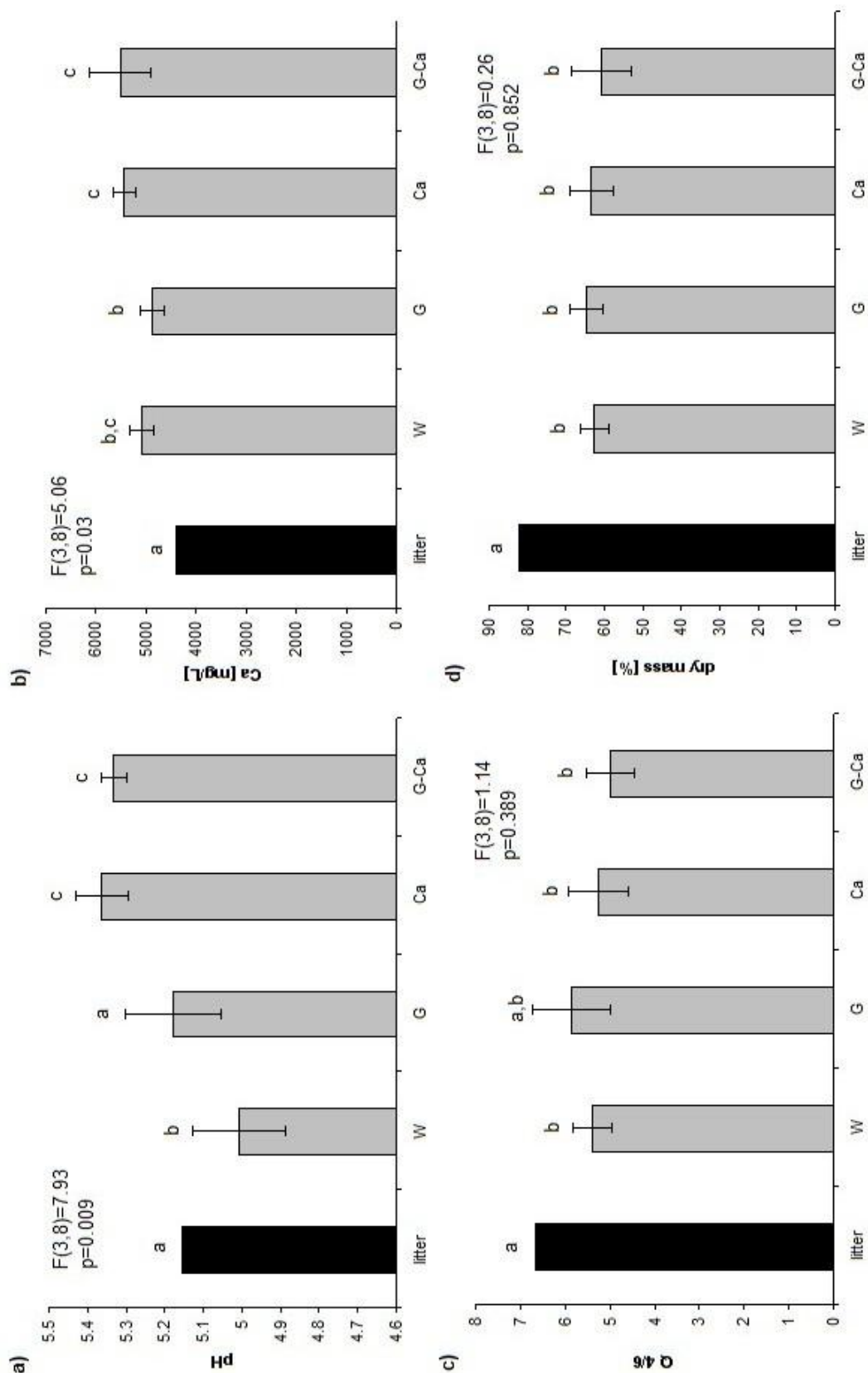**Figure 4:** Effects of different treatments on pH (a), Ca concentration (b), Q 4/6 (c), and dry mass (d) of the litter. Treatments are water (W), glucose (G), CaCO3 (Ca), and glucose and CaCO3 (G-Ca). There are also shown characteristics of the original litter for comparison. Columns signed with the same letters are not significantly different. GLM, Post-hoc test Fisher LSD (mean and confidence intervals are shown).

Quotient Q 4/6 and dry mass were not significantly affected by glucose and $CaCO_3$ treatments (Q 4/6 $F_{(3,8)}=1.14$, p=0.389; dry mass $F_{(3,8)}=0.26$, p=0.852) (Fig. 4c,d), but both properties in all treatments decreased in comparison to the original litter.

# 5. Discussion

As we expected, glucose and $Ca^{2+}$ cations had significant effects on increase in pH and can be assumed as the major reason for pH increase in wood ant nests reported in previous studies (Dlusskij 1967; Frouz and Jílková 2008). Basic cations, such as $Ca^{2+}$, $Mg^{2+}$, $K^+$ and $Na^+$, are known to increase pH due to their ability to act in the cation exchange complex (Brady and Weil 2002; Šimek 2005). Addition of simple sugars, especially glucose, in form of honeydew into the soil activates microorganisms and increases their metabolic activity and thereby decomposition processes (Joergensen and Scheu 1999; Seeger and Filser 2008; Stadler et al. 1998). Due to these processes many mineral compounds are released from organic materials such as coniferous needles, twigs and leaves, as well as from food of ants (Brady and Weil 2002; Edwards et al. 1970; Paul and Clark 1996). Basic cations in mineral compounds are then responsible for increase in pH (Brady and Weil 2002).

Amounts of glucose and $Ca^{2+}$ cations used in this study are sufficient to significantly increase pH of the litter. According to our study, ants bring about 16 kg of dry mass of honeydew into their nests per year. This amount corresponds to the lower limit of the range of amount of honeydew brought into ant nests found by Frouz et al. (1997) and also corresponds to the amount of glucose used in the manipulation experiment, if we take into account that mounds in this study were smaller than regular nests are. As aphids produce honeydew in large quantities (Stadler et al. 1998), ants are able to consume about 80% of honeydew (Rosengren and Sundström 1991). The rest reaches the forest floor and there acts as a bottom-up force for soil organisms. Honeydew consumed by ants is transported into ant nests where it is redistributed and utilized as a source of energy (Horstmann 1974). Ants need to utilize only about 90% of honeydew brought into the nest, the rest is then passed to soil organisms (Frouz

et al. 1997). This part of honeydew encourages microbial activity and decomposition processes in ant nests.

Ants are able to bring large amounts of basic cations in their food and building material for their nests. Contents of basic cations brought into the nest in honeydew and prey reach up to 272 g of $Ca^{2+}$, 42 g $Mg^{2+}$, 240 g $K^+$ and 39 g $Na^+$ cations annually. These amounts are larger than amounts of basic cations released from the litter. According to Pokarzhevskij (1981), 12-37% of nest material is brought by ants into the nest annually. This comprises about 35 kg of the needle litter (Frouz et al. 1997). 50% of this organic material is decomposed in ant nests. $Ca^{2+}$ cations are represented by 0.55%, $Mg^{2+}$ cations by 0.12%, $K^+$ cations by 0.22% and $Na^+$ cations by 0.13% in Norway spruce litter (Pedersen and Bille-Hansen 1999). The amounts of basic cations released from the litter in ant nests reaches roughly 100 g for $Ca^{2+}$, 21 g for $Mg^{2+}$, 40 g for $K^+$ and 23 g for $Na^+$ per year. Both these contributions of basic cations together are responsible for the change in pH in ant mounds.

Phloem sap is alkaline (pH 7.8 to 8.4), the principal dissolved cation is $K^+$, little $Ca^{2+}$ is dissolved in phloem sap (Fahey and Birk 1991). $K^+$ cation is also the mobile one, whereas $Ca^{2+}$ cation is immobile. This could explain higher contents of $K^+$ cations than $Ca^{2+}$ cations brought in the honeydew into ant nests. Contents of basic cations in ant prey collected in this study are similar to those found by Ghaly (2009).

$Ca^{2+}$ cations have significantly stronger influence on increase in pH of ant nests materials than glucose. This could be caused by their direct action in the cation exchange complex (Brady and Weil 2002), whereas glucose acts only as a priming effect for mineralization processes performed by microorganisms (Joergensen and Scheu 1999). Another explanation suggests that $Ca^{2+}$ cations cause increase in pH that in turn provides more suitable conditions for decomposition as the optimum for most microorganisms occurs in neutral pH (Brady and Weil 2002; Edwards et al. 1970; Steubing 1970). Basic cations, not only $Ca^{2+}$ but also $Mg^{2+}$,

$K^+$ and $Na^+$, are released through these decomposition processes and thus pH of the litter is even more increased. This explanation is also supported by amounts of $Ca^{2+}$ cations that are not significantly increased in Ca and G-Ca treatments unlike significantly increased pH in these treatments suggesting that there are also other effects on increase in pH. Decomposition proceeds also in untreated litter, which is showed by increased amount of $Ca^{2+}$ cations in W treatment.

In W treatment, pH of the litter decreased in comparison to the original litter. This decrease is most likely caused by leaching of basic cations from the litter by rain water, which commonly occurs in temperate forest soils (Brady and Weil 2002; Edwards et al. 1970). In G, Ca and G-Ca treatments, the amount of leached cations is compensated either by addition of $Ca^{2+}$ cations or by addition of glucose encouraging release of basic cations from the litter.

Litter in all treatments showed lower quotient Q 4/6 in comparison to the original litter suggesting humus acids to be more stable and polymerized due to decomposition processes (Valla 2000). Higher polymerization of humic acids in comparison to fulvic acids can also contribute to the lower acidity of the material (Brady and Weil 2002) and hence to pH increase in wood ant nests.

Dry mass in the mounds was found lower in comparison to the original litter. This is not a surprising result given that ants were absent from our mounds and thus low moisture was not maintained due to ant activities. Ants are able to keep their mounds dry by several mechanisms, e.g., by maintaining a surface layer that prevents rain water from infiltration into a mound, or by building chambers and corridors in their nests, which improves aeration and evaporation of water from the nest material (Lafleur et al. 2002; Petal 1978).

Our results show that biogeochemical processes in wood ant nests triggered by input of food, namely by input of basic cations and easily decomposable carbon, may cause substantial increase in pH and can be assumed as the principal mechanism responsible for pH increase

observed in previous studies (Dlusskij 1967; Frouz and Jílková 2008). However, other mechanisms suggested in previous studies, namely soil mixing by *Lasius niger* (Frouz et al. 2003), may also contribute to pH increase in the soil rim of wood ant nests especially.

# 6. Conclusion

Wood ants due to their activities influence many soil properties, among others pH. They do this through their foraging and constructing activities. Ants are able to bring large amounts of food and building material into their nests annually. Decomposition processes in ant mounds are enhanced as a consequence of favourable microclimatic conditions and input of simple sugars, such as glucose, in honeydew. The amount of glucose imported in honeydew into ant nests is responsible for the increase in microbial activity and in turn for increased contents of basic cations released from organic materials, building material and food of ants. This effect together with amounts of basic cations imported into ant nests in food and building material is most likely responsible for the increase in pH which is commonly found in ant nests.

# 7. References

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# 8. Appendix

Jílková V, Matějíček L and Frouz J (2011) Changes in the pH and other soil chemical parameters in soil surrounding wood ant (*Formica polyctena*) nests. Europen Journal of Soil Biology 47: 72-76.

Abstract:

Several previous studies reported on how the chemistry of the wood ant nest differs from the chemistry of the surrounding soil. There is enhanced amount of nutrients and cations in the ant nest and pH also differs from the nest surroundings. In this contribution, we focused on changes in soil chemistry with distance from the nest. Samples of mineral soil 0‑5 cm deep were taken in grid pattern at 1‑19 m from six *Formica polyctena* nests in a spruce forest in the Czech Republic.

Soil pH decreased with distance; pH decreased rapidly between 1 to about 8 m and then decreased more slowly. The decrease in pH corresponded with the increase in organic matter content with distance from the nest. Organic matter content was significantly and positively correlated with available Ca and K, i.e., available Ca and K increased with distance from the nest. The changes in organic matter content, pH, and available Ca were caused by ants collecting and using needles as building material. Wood ants can affect soil properties by rearranging organic matter, in nest surroundings.