I. CARBON DIOXIDE CONCENTRATIONS IN THE NESTS OF THE LEAF-CUTTING ANT *ATTA VOLLENWEIDERI*

Nest mound of *Atta vollenweideri* in the palm savanna of Gran Chaco (Argentina)

Introduction

Most species of social insects build nests that protect the colony and especially the brood against predators and provide a microclimate suited to the colonies needs. The major microclimatic variables regulated by a colony are temperature, humidity and the concentration of respiratory gases. Colony members react to changes in microclimatic conditions at two different levels. Following short-term fluctuations, e.g. in temperature during the day, behavioral responses such as fanning, water collection for cooling or shivering to produce heat can be observed (Seeley and Heinrich 1981). Long-term reactions to unsuitable conditions are mainly activities that lead to modifications of the nest structure (Korb and Linsenmair 1999). Nest building and control of nest microclimate assure brood development and ultimately colony growth (Vogt 1986).

Microclimatic variables can be independently regulated only within a limited range. Regulation of one parameter to its optimum will often drive another parameter to its suboptimum. For example, nest temperatures higher than ambient temperature require some kind of nest insulation and can thus often be achieved only at the expense of gas exchange (Seeley and Heinrich 1981; Hölldobler and Wilson 1990). A colonies humidity demands can also conflict with the necessity for gas exchange (Scherba 1958; Collins 1969; Noirot 1970). Tradeoffs in regulation of microclimatic conditions are therefore expected to occur, and the insect society has to balance its efforts accordingly.

Gas exchange between nest and environment is essential. Due to colony respiration considerable amounts of O_2 are consumed and CO_2 produced. In closed dwellings this leads to hypoxic and hypercapnic conditions, as is also observed in nonsocial insects (Howarth 1983; Anderson and Ultsch 1987). Several investigations report CO_2 concentrations ranging from 0.05% (normal) to 15% in nests of social insects (Lüscher 1956; Es´Kov 1974; Matsumoto 1977; Anderson and Ultsch 1987; Kirchner 1998).

The influence of CO_2 on insects has been thoroughly investigated and reviewed (Nicolas and Sillans 1989). Various physiological effects have been reported, e.g. changes in acidity of the haemolymph and in hormone titers (Pasche and Zachariassen 1973; Bühler *et al*. 1983; Röseler and Röseler 1984).

With the exception of termites little is known about the regulation of respiratory gases in the nests of social insects (Lüscher 1961; Seeley 1974). Colonies of the leaf-cutting ant *Atta vollenweideri* contain up to several million individuals (Weber 1972). Mature colonies are expected to produce large amounts of CO_2. Without nest ventilation, CO_2 production will be associated with a proportional reduction in the availability of O_2 due to the respiration of both fungus and ants in the limited space of the nest chambers. Hypoxic and hypercapnic conditions may affect colony respiration, and the existence of active and/or passive mechanisms for nest ventilation is expected. It is important to note that while the ability to perceive CO_2 is widespread in insects, no biological system is known to sense the O_2 of the ambient air and that therefore CO_2 is the biologically relevant parameter providing the ants with indirect information about the essential O_2 availability. Leaf-cutting ant workers possess the ability to measure not only the relative but also the absolute CO_2 concentrations with special sensory organs, an unusual feature that points to the fact that the information about actual CO_2

concentrations is of great importance for the ants and that will be discussed in detail in chapter five.

During the rainy season the habitat of *Atta vollenweideri* is regularly flooded and flooding often confines the ants to their nests and prevents them from foraging for plant material (Jonkman 1979). While mounds of mature colonies protect colonies from excessive water influx because all the nest openings are located more than 20 cm above the surrounding, small developing colonies are vulnerable to flooding due to the small size of their mounds. Thus, when facing strong precipitation and flooding, they tightly close all nest openings with twigs and clay crumbs (Jonkman 1980). This situation often needs to be maintained for several days and colony members as well as the fungus are exposed to increasing concentrations of CO_2 and decreasing concentrations of O_2. Protection of the colony against water influx constrains nest ventilation and could consequently affect colony respiration.

The aim of the present study was to quantify CO_2 concentrations inside field nests of *Atta vollenweideri* and to assess the influence of nest ventilation on CO_2 concentrations inside the nest chambers. Furthermore, the potential tradeoff between protection of the nest against water influx by closing of nest openings and the need of gas exchange in small colonies was examined.

The enormous colony size, the uniform architecture of the mound and the well known arrangement of the subterranean nest chambers (Eidmannn 1935; Daguerre 1945; Jonkman 1980) make *Atta vollenweideri* an excellent object for the study of climatic nest conditions.

Methods

The field experiments were done in the National Park Río Pilcomayo, Formosa Province, Argentina (58°W; 25°S). Measurements were performed in different nests during December of 1997 to February of 1998 (across weather conditions). The annual average temperature in this region is 23°C and the mean precipitation is 1200 mm (mean relative humidity 79%). Precipitation exceeds evaporation in all months of the year except in August (Pujalte *et al*. 1995).

Nest characteristics

The size of the nest mound can be used as criterion for maturity of a colony. Once clearly established, a nest of the leaf-cutting ant *Atta vollenweideri* reaches an average age of about 10 years (Jonkman 1978; Jonkman 1980). In order to assess the influence of nest size on CO_2 concentration and nest ventilation, different nests representing the extremes in the size range observed in the research area were chosen. Seven mature nests and one small nest were investigated. Even though several small nests were found, for some of them a clear identification of their age based on mound size was not possible. A small mound could correspond either to a young nest or to an old nest that partially collapsed.

Since the internal structure strongly depends on nest age, it was crucial to perform measurements in small nests of known age. This was possible only for one small nest which had been identified in a previous field trip.

Nest volume was calculated using either the formula for a cone ($V = \pi r_1 r_2 h/3$) or a dome (sphere segment) ($V = \pi h(3r_1 r_2 + h^2)/6$) depending on the shape of the mound. The height (h) of the nest was defined as the difference between ground level and top of the mound. Ground level was considered as the level of the surrounding area of the nest. The radii (r_1;r_2) were half of the largest and smallest diameter of the nest, respectively.

The mound of the small nest was described by mapping the heights with a grid of 20 cm. The volume was calculated by adding all cuboids. The area of nest openings was calculated using the cross section of the channels measured at the narrowest part in the first 10 cm from the openings with the formula $A = \pi r_1 r_2$. Several investigations were made in one mature and one small nest. The same two nests were used and are referred to in the text as mature (M) and small nest (S).

Microclimate in the nests

The concentration of CO_2 was measured by IR-absorption with a gas sensor type GS 20 ED/CO2 (Sensor Devices, Germany). Data were logged with a self-made data logger, equipped with a micro computer (Tiger Basic), and transferred to a laptop for processing.

Air samples were collected by drilling an iron pipe 1.5 m into the mound. The pipe could be prolonged for another 1.5 m, thus air samples from up to 3 m below surface could be taken. Air probes were sucked from different depths of the nest with a membrane pump (12 V Wisa, Germany) for 3 min with a flow rate of 1.5 $lmin^{-1}$. During long term-recordings air probes were taken every 3 hours.

The effects of wind on the CO_2 concentration inside the nest were investigated by performing simultaneous long-term recordings of wind velocity and CO_2 concentration. The measurements were performed on 20/21 February. The wind velocity was qualitatively indicated by the excursion of a cotton filament and four categories were defined. Zero indicated no-wind and the category 3 corresponded to approx. 1 ms^{-1}. During CO_2 data collection, the filament was observed for 5 min and the wind velocity was assigned to one of the categories.

In order to investigate the influence of environmental temperature and humidity on nest microclimate, they were recorded during a hot and a dry weather period. Measurements were always taken during the afternoon to allow comparison. Humidity and temperature were recorded with two testing probes (Vaisala HMP 36B) and logged with a Vaisala HMI 36 data processor. On 9 December two testing probes were introduced via the biggest channel in the center of a mature nest (M) at 1 m and at 0.1 m, respectively. Data were logged every hour for 24 h. On 26 December humidity and temperature were measured in different channels of the mature nest and the small nest at a depth of 40 cm in the afternoon (14:00-16:00 h with similar weather conditions as on 9 Dec.). Wind velocity during daytime was at least 3 ms^{-1}.

Respiration rate of small colonies

The consequence of reduced exchange of respiratory gases on colony respiration was investigated in a small laboratory colony of *Atta sexdens*, used as a model for the respiration of *Atta vollenweideri*. Even though *Atta sexdens* and *Atta vollenweideri* may indeed have the same species of fungal symbiont (*Attamyces bromatificus*), it has been suggested that due to the clonal nature of the reproduction of the fungus, species-specific fungal strains are likely to evolve quickly (Stradling and Powell 1986; Chapela *et al*. 1994; Mueller *et al*. 1998). However, since both *Atta* species construct very large nests and probably face similar conditions regarding the concentration of respiratory gases inside the chambers, it appears to be unlikely that particular differences in respiratory physiology between fungal strains may have evolved. But because this assumption cannot be supported with data, the results on *A. sexdens* should only illustrate the general effects of CO_2 concentration on colony respiration by leaf-cutting ants.

The experiments were done in Würzburg during the summer of 1998, using a laboratory colony of *Atta sexdens* collected in Botucatú, São Paulo, Brazil. The colony was about 3 years old and the fungus garden occupied a volume of about 12 l. The colony was reared at 25°C and 50% relative humidity in a 12h/12h photoperiod and fed predominantly with leaves of privet (*Ligustrum vulgaris*).

The CO_2 production of the tightly enclosed colony was measured over time in order to determine colony respiration rate as a function of increasing CO_2 concentration. If there is no gas exchange with the environment and colony respiration is not affected by the CO_2 concentration, a linear increase of CO_2 concentration (i.e., constant respiratory rate) with time would be expected.

One-half of the colony (two boxes) was used in all experiments. It was kept in a closed-loop system for either 2 or 3 days in which the volume occupied by the fungus decreased from 4.3 l to 3.1 l. The closed-loop system simulated the conditions of a small colony during a rainy period when all nest openings are closed. The total volume of the closed-loop system was 11.3 l composed of a plastic box with a tube connection to a CO_2 analyzer and a pump. 0.4 l of clay crumbs saturated with water were added to the plastic box. By continuously pumping air at a rate of 1 $lmin^{-1}$ through the closed-loop system, air was mixed in the box and renewed in the measuring chamber of the CO_2 analyzer. In all experiments the CO_2 concentrations inside the closed system were measured every 5 min for 160 min. The experiment was repeated 6 times and all data were pooled.

The rate of CO_2 absorption by the material of the closed-loop system or by dilution in water was determined in control experiments. The colony was removed from the boxes and the CO_2 concentration in the empty nest was artificially increased by injecting pure CO_2 (a valve was added to avoid hyperpressure). Injection was done with a magnetic valve (Lee Company, USA) every 5 min for 4 s with 3 different pressures, resulting in the following flow rates: 246 mlh^{-1} (SD ±13.34 ml; n = 3), 326 mlh^{-1} and 380 mlh^{-1} CO_2. These flow rates were in the range of the CO_2 production rate of the investigated colony. Data were captured using the same protocol as described above. The injected CO_2 volume was calculated by multiplying the difference in CO_2 concentration between starting point and after the first hour with the total volume of the closed-loop system (10.9 l).

Results

Microclimate in the nests

Morphology of the nest mound and CO_2 concentrations at different depths are summarized in Table 1.1 for the investigated nests. In mature nests the measured CO_2 concentration was significantly lower at a mean depth of 0.61 m below the normal level than at a depth of 2.07 m ($T_+ = 28$, $p < 0.01$; Wilcoxon matched paired (Siegel and Castellan 1988)). There was a high variability in CO_2 concentration at both depths (upper depth: 0.78% CO_2; SD ±0.48; lower depth 1.94% CO_2; SD ±0.77; n = 7). No significant correlation between mound size and CO_2 concentration could be found in mature nests (upper depth: Spearmans R = -0.52; p = 0.29; lower depth: Spearmans R = -0.26; p = 0.62). In the small nest the mean CO_2 concentration was 2.44% CO_2 (SD ±1.46; n = 23) in repeated measurements on different days.

Shape of mound	Volume (m^3)	Height (m)	Upper depth (m)	[CO_2] in upper depth (%)	Lower depth (m)	[CO_2] in lower depth (%)
dome	16.1	1.0	0.5	0.83	2.0	1.96
cone	10.7	1.0	0.7	1.08	2.2	2.74
dome	20.2	0.9	0.5	0.07	2.0	1.10
dome	59.8*	0.9	0.4	0.29	1.9	2.50
dome	10.9	0.7	0.8	0.83	2.0	1.59
dome	9.3	0.6	0.9	1.53	2.4	2.79
cone	12.1	1.0	0.5	0.81	2.0	0.93
mean	**13.2**	**0.87**	**0.61**	**0.78**	**2.07**	**1.94**
Std.Dev	4.1	0.16	0.19	0.48	0.17	0.77

Table 1.1 Shape and CO_2 concentration at two different depths of seven mature nests measured on different days. Height: Elevation above ground level.
* Not taken into account for statistics because mounds of two adjacent nests probably merged.

The measurements that are reported in the following paragraphs were taken on one small and one mature nest, which represent the extremes in the size range observed, and are expected to provide the maximum difference in the variables under scrutiny.

The influence of wind on the CO_2 concentration inside the nests was investigated by performing parallel long-term recordings in the mature (M) and the small nest (S). There was a strong negative correlation between wind velocity and CO_2 concentration in both nests (Fig.1.1). In the mature nest the correlation coefficient between wind velocity and the CO_2 concentration was R = -0.82 at 2 m ($p < 0.05$; Spearmans R), and R = -0.85 at 0.5 m ($p < 0.05$; Spearmans R). In the small nest it reached R = -0.92 ($p < 0.01$; Spearmans R). The CO_2 concentration increased during no-wind conditions (local time 18:00, Fig.1.1) in both nests.

The influence of foraging activity or ant activity in the nest on the CO_2 concentration was not determined but presumably they have little influence on the variability of the CO_2 concentration as foraging activity was different in the two parallel observed nests.

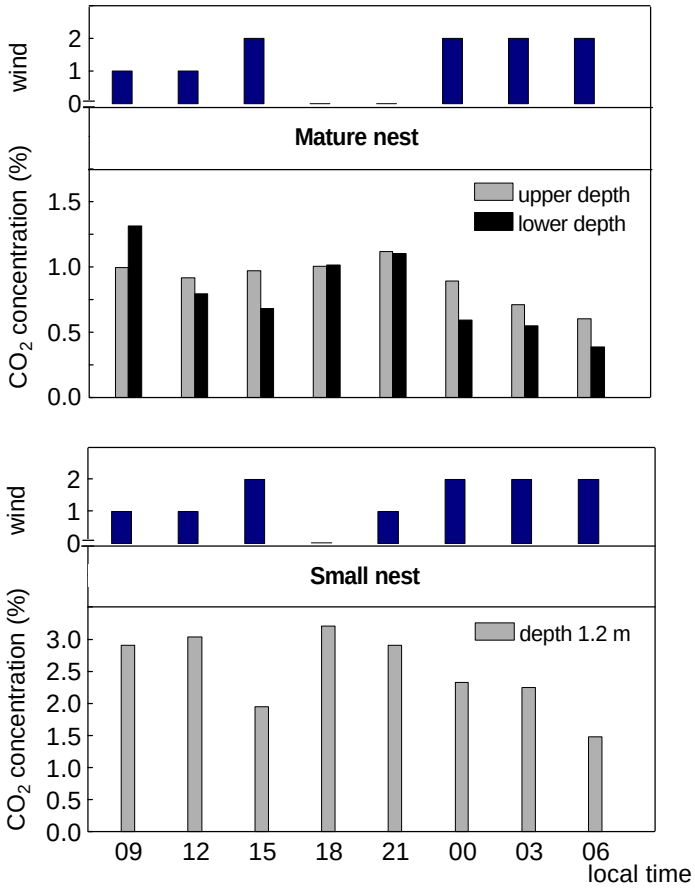

Fig.1.1 Wind velocity (in categories) and CO_2 concentrations during 21 h in two different nests. Mature nest (upper) with a mean CO_2 concentration of 0.80% at a depth of 2.0 m and 0.90% at 0.5 m. Small nest (lower) with a mean CO_2 concentration of 2.50% at a depth of 1.2 m. Note that any increase in wind velocity is followed by a decrease in the CO_2 concentration inside the nest and vice versa. The CO_2 concentration was measured during 21 hours every 3 hours in a mature nest and with a delay of 20 min in the small nest.

Inflowing air might change the internal nest microclimate concerning temperature and humidity. Fig.1.2 shows as an example the daily course of relative humidity and temperature at two different depths in the mature nest (M). In a channel at a depth of 1 m below zenith of the dome the temperature and the relative humidity were constant. The mean temperature was 27.5°C (range: 27.4-27.6°C) and the mean relative humidity was 95.9% on this day. Just below the surface of the mound, 0.1 m down in a channel the temperature and relative humidity varied with the daily temperature changes in the savanna. The mean temperature was 33.7°C (range: 28.0-40.9°C) with a mean relative humidity of 70.6% (range 43.4-91.3%). In the environment, the mean daily temperature was 31.0°C (range: 24.4-36.1°C) 1.5 m above ground in the shadow, with a mean relative humidity of 71.2% (range: 52.0-93.2%). Thus, in the nests measured ventilation did not influence humidity and temperature inside the fungus chambers.

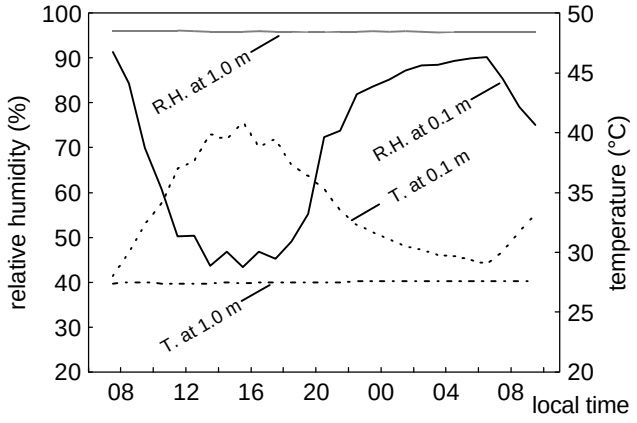

Fig.1.2 Example of the daily course of relative humidity and temperature in a mature nest. Testing probes were inducted into a channel at two different depths, 1.0 and 0.1 m. Note the constant relative humidity and temperature at the lower depth. At 0.1 m, the temperature and the relative humidity followed the daily environmental changes.

Since it was not possible to measure temperature and humidity in a depth of 1 m inside the small nest, these parameters were measured at a depth of 40 cm in different channels of both nests. In the mature nest (M) the temperature was 33.2°C (SD ±1.90; n = 12) with a relative humidity of 76.0% (SD ±15.16; n = 12). In the small nest (S) in all of the 6 visible channels the temperature was 31.5°C (SD ±0.3; n = 6) with a relative humidity of 93.3% (SD ±4.36; n = 6). Note that the variance of data is much higher in the channels of the mature than in the small nest, which had a higher relative humidity, indicating that a greater volume of dry air is flowing through the channels of the mature nest.

Nest closure of small colonies

The small nest was investigated in greater detail since small colonies have the demand of nest closure during strong precipitation and flooding.

The occurrence of strong precipitation during the rainy season is, a priori, one important factor influencing nest microclimate. Different behavioral reactions to strong precipitation were observed between small and mature colonies. Several small colonies were observed to react to heavy rain by closing all nest openings. The smallest nests observed (with only one or two entrances) were often completely closed even if protection against flooding seemed to be unnecessary. Building activity at the openings of mature nests was not very pronounced during heavy rain, even though eager transportation of clay crumbs out from the dwellings could be observed after rain in all nests.

The influence of enclosure on the nest microclimate was assessed by measuring CO_2 concentrations in the small nest (S) over a period of 3 days. The nest openings were closed by the ants due to heavy rain in the first hours of the second day (20.02). The ants occluded the nest channels by covering the openings with twigs. Water-soaked clay crumbs tamped the remaining cracks. It took only about 1 h until all 6 openings were closed and about the same time after rain had ceased for reopening them, though to a smaller aperture. Unfortunately the testing probe was also occluded by the ants activity. It had to be removed and was reinserted at the same spot as before.

Occlusion of all nest openings led to a steep increase of CO_2 concentration inside the nest (Fig.1.3). Over 24 h the CO_2 concentration rose from 1.09% to 5.69% (20.02). Therefore the rate of increase in CO_2 concentration on this day was 0.19% CO_2h^{-1}. The total aperture (cross section of all 6 channels summed) was 114 cm^2 at the beginning (19.02; 00:00). During rain all channels were completely closed (20.02; 01:00-07:00). Eight hours later the total aperture was 56 cm^2 (20.02; 15:00). No-wind condition during the following hours might have contributed to the further increase of CO_2 concentration. On the following day the total aperture was 92 cm^2.

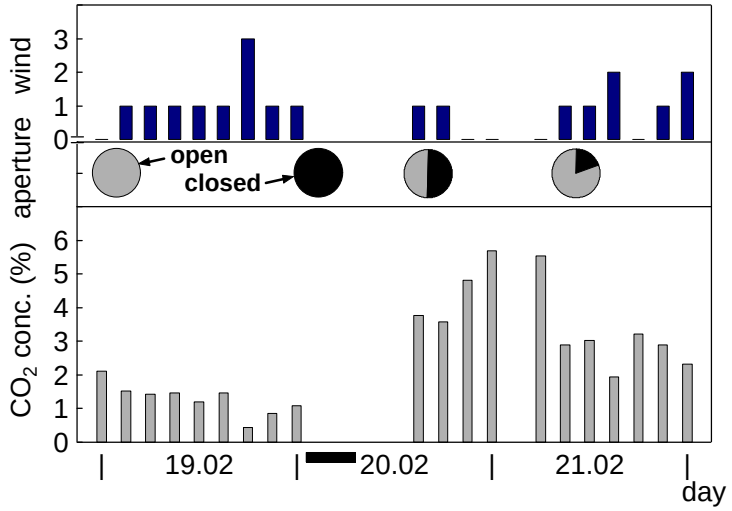

Fig.1.3 Wind velocity (upper, in categories) and aperture of all nest openings (pie chart; middle), and their correlation with CO_2 concentration at a depth of 1.2 m (lower) in the small nest over a period of 3 days. The horizontal bar on the x-axis indicates heavy rain over 6 h with 50 mm precipitation. No data could be taken on 20 Feb. 03:00-12:00 and 21 Feb. 03:00.

The conditions prevailing during heavy rain were simulated in an independent experiment by tightly occluding all openings of the small nest with the lower half of plastic bottles. Before occlusion only very few foragers were outside the nest. They were stimulated to return into the nest by irrigating them with water and thus simulating rain. The occlusion disturbed the colony only a little at the beginning. As soon as condensed water dropped from the inner wall of the plastic covers, the ants started to close the openings. Fig.1.4 shows the time-course of CO_2 concentration rate, which was observed to rise linearly from 1.88% CO_2 after 30 min of total occlusion to 3.75% CO_2 after 6 h. The increase in CO_2 concentration averaged 0.30% CO_2h^{-1}, which lies in the range of that observed under ant-made occlusion of all openings.

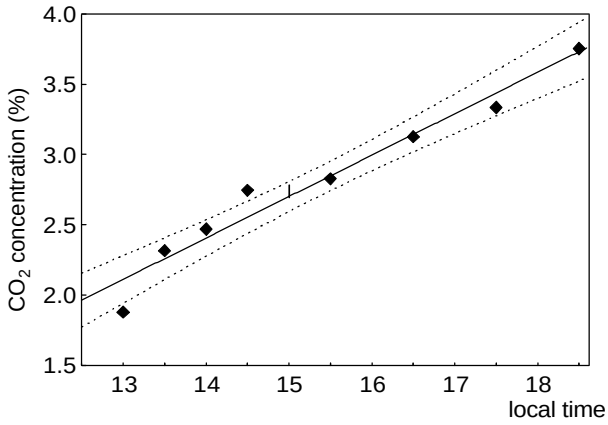

Fig.1.4 Increase of CO_2 concentration in the small nest after artificial occlusion of all openings at 12:30 local time. Linear regression with 95% confidence interval of the slope (dotted line). Air samples for CO_2 analysis were taken over six hours (sampling rate: 30 min in the first 3 hours; 60 min in the second 3 hours).

Respiration rate of a colony

A closed-loop system was used to investigate the effects of increasing CO_2 concentration on colony respiration. Expected and measured colony-induced CO_2 increase are presented in Fig.1.5. There was a clear reduction in CO_2 accumulation rate in the closed-loop system as a function of increasing CO_2 concentration. Any decrease in CO_2 accumulation rate with time indicates either a CO_2-induced reduction in colony metabolism or a loss of CO_2 in the gas phase of the system (buffer capacity). In order to control for any CO_2 sink, the CO_2 concentration was artificially increased by injection into an empty nest (see methods). A slight decrease of the CO_2 accumulation rate could be measured (Fig.1.5: injection), indicating a sink of CO_2 in the system which needs to be taken into account in the calculations.

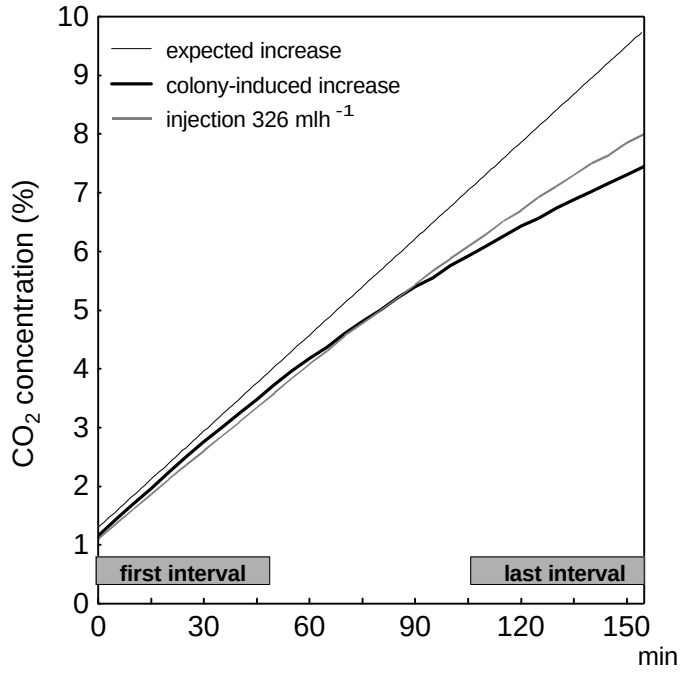

Fig.1.5 Time-course of increasing CO_2 concentration in a closed-loop system. Any decrease in CO_2 production or accumulation rate is expressed by a shallower slope. Measured example: Colony-induced increase and increase by injection. Theoretical example: Expected increase at a fictive CO_2 production rate of 3.4% CO_2h^{-1}. Horizontal bars indicate the intervals used for calculating the CO_2 production rates shown in Fig.1.6.

The mean CO_2 production rates of the colony as a function of CO_2 concentration are presented in Fig.1.6. Two different concentration ranges were defined in order to clarify the presentation of the data. The two concentration ranges correspond to two time intervals, 50 min each and indicated as horizontal bars in Fig.1.5. In the experiment with the colony the mean CO_2 concentration at the beginning was 1.17% CO_2 (SD ±0.14; n = 6) and at the end 7.31% CO_2 (SD ±0.62; n = 6). The rate of CO_2 production in the first 50 min (from 1.17-3.39% CO_2) was 2.98% CO_2h^{-1} (SD ±0.38; n = 6) and in the last 50 min (from 5.85-7.31% CO_2) it was 1.74% CO_2h^{-1} (SD ±0.06; n = 6) (Fig.1.6; left and lower right).

In the control experiment with an empty nest, mean CO_2 concentration at the beginning was 1.14% (SD ±0.05; n = 5). Irrespective of the flow rate used a reduction of 0.45% CO_2h^{-1} (SD ±0.06; n = 5) was found in the last 50 min before reaching the 7% level. Therefore 0.45% CO_2h^{-1} was used for correction of the colony-induced CO_2 production rate in the last 50 min (error and deviation added). This value is represented in Fig.1.6 (upper right).

Compared to the CO_2 production rate of the colony at the beginning (2.98% CO_2h^{-1}) the resulting production rate at the end of the experiment (2.18% CO_2h^{-1}) represents a reduction of 27%.

The calculated rate of CO_2 production by the colony was 273 mlh^{-1} at low CO_2 concentrations (from 1.17 to 3.39% CO_2), and 160 mlh^{-1} at high concentrations (from 5.85 to 7.31% CO_2).

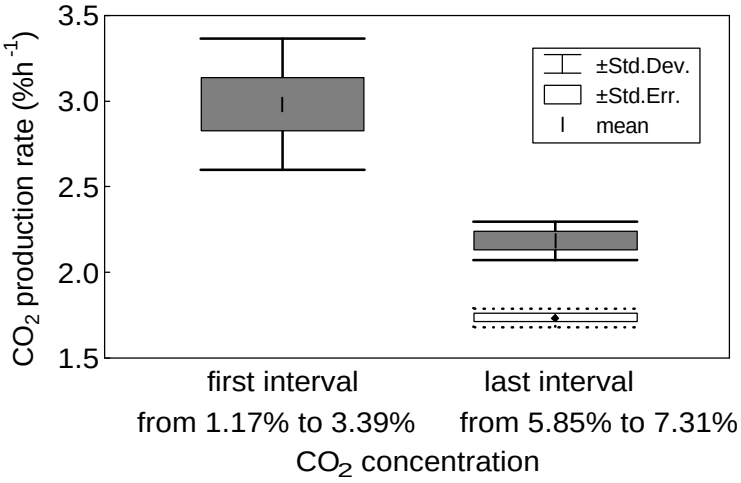

Fig.1.6 CO_2 production rate of a small colony over 160 min in a closed-loop system. The CO_2 production rate was calculated for the first interval shown on the left side (first 50 min) and for the last interval on the right (last 50 min, see Fig.1.5). The dotted box/whisker (bottom right) indicates the CO_2 production rate before correction with a factor of 0.45% CO_2h^{-1} for the buffer capacity of the system.

Discussion

CO_2 concentrations

The architecture of the nests allows a distinction between a living area with fungus chambers and an area with dump chambers. The fungus chambers are located above the dump chambers and have never been found at depths greater than 2 m (Jonkman 1980). Therefore the air probes from the upper part of the mature nests can be considered as probes from the area with fungus chambers and those from the lower part as probes from the area with dump chambers.

In the area with fungus chambers, the CO_2 concentration with a mean of less than 1% CO_2 was astonishingly low. In the area of the dump chambers it was higher, with a mean of 2% but still lower than expected. This indicates that mature nests are generally well ventilated, irrespective of the variability in nest shape and wind conditions in between measurements.

The high variability of the measured CO_2 concentrations probably reflect the fact that the probe could not be selectively inserted into a chamber, and that thus some air probes might have been taken from channels connecting the chambers.

Ventilation

CO_2 concentration was used as a measure for ventilation because it allowed the measurement of gas concentration inside the nest without destroying any nest structures. The hole which was drilled into the nest was only 14 mm wide and small injuries to the nest are normally quickly repaired by the ants. Measurement using IR-absorption is fast, only small amounts of air have to be sucked from the nest and ventilation is thus influenced as little as possible.

Wind was observed to induce the exchange of air in the channels of the nest. In both nests the strong negative correlation between wind velocity and CO_2 concentration impressively illustrates the significance of wind as the driving-force for ventilation. At least two physical principles may underlie wind-induced ventilation. Both make use of Bernoulli's principle of pressure differences induced by differences in flow velocity which can be used to drive a secondary flow (Vogel 1994). The mechanisms of wind-induced nest ventilation, the role of the channels and the contribution of thermal convection for nest ventilation, which has been proposed by several authors (Weber 1972), will be discussed in detail in chapter two.

Temperature and humidity

Although the air in the channels near the surface and 40 cm into the ground was hot and of low relative humidity in some of the channels, the daily fluctuations of these parameters were not conveyed into the nest. Solar radiation heated the upper parts of the mound but inflowing air was cooled down, presumably due to the great surface area in the channels and the high water content of the soil. Temperatures in channels 1 m below surface were never above 30°C, confirming the results of previous investigations in nests of *Atta* (Weber 1959).

The relative humidity in the nests was always above 90% at a depth where fungus chambers occur, so that the ventilation demands of the colony during summer do not compromise the humidity conditions inside the nest. The conditions for optimum growth of the Attine symbiont fungus are condensing humidity, temperatures of about 25°C and an acidity of 4.5-5.0 pH (Quinlan and Cherrett 1978; Powell and Stradling 1986). Temperatures higher than 30°C are lethal for the fungus, so that inflowing air has to be cooled down in summer, when air temperatures is above 40°C.

No data are available to decide whether 30°C is a suboptimal temperature for leaf-cutting ant workers as it is for other ant species. In general ants are strongly thermophilic organisms (Hölldobler and Wilson 1990; Heinrich 1993). Lacking wings, ants are not able to ventilate their nests by fanning like bees or wasps, nor are they able to heat up the nests by shivering. Therefore ants have to rely on the correct location of the nest, an efficient construction, migration within the nest (Roces and Núñez 1989; Roces and Núñez 1995), and on the use of metabolic heat by clustering or dispersing. How successful leaf-cutting ants are in incubating their fungus garden was shown for *Acromyrmex ambiguus* in the Argentine pampa where the garden was heated up to more than 10°C above soil temperature (Weber 1972). Suboptimal temperatures primarily inhibit or reduce brood development, which directly influences the growth rate of a colony (Himmer 1927; Porter 1988). Reducing the number of openings and thereby reducing ventilation during winter might lead to an increase of nest temperature in mature nests, an aspect which remains to be shown.

For *Atta vollenweideri* with the southernmost distribution within the Attini, nest temperature might be a decisive factor for colony growth in winter when environmental temperatures are below 17°C (mean in August). With restricted temperature tolerance and high relative humidity demands, the *Atta* symbiont appears to set the limits within which optimal colony growth rates can be achieved.

Nest closure of small colonies

Heavy rain in summer induces a conspicuous reaction in small colonies, namely occlusion of all nest openings. During the growth phase small nests are not protected against flooding because of their low height. Therefore a young colony has to close all openings and to sustain occlusion of the nest for several days. Small nests are usually flat at the top and following rain puddles can often be observed for several hours above former openings. In contrast, the shape of mature nests facilitates the drainage of water. Thus, precipitation represents a stronger stress factor for small than for mature colonies. While small colonies have to handle the tradeoff between water influx and ventilation during rainy periods, mature colonies do not need to close the nest openings in this situation. Enclosure of the dwellings leads to an immediate increase of CO_2 concentration inside the nest. As a consequence the O_2 concentration decreases to an extent that depends on the respiratory quotient (RQ) of the involved participants.

It has been shown that respiration of the ants (workers) is characterized by lipid metabolism (Lighton *et al*. 1987), whereas the fungus metabolism is based on carbohydrates. Postulating a RQ = 1 for the whole colony, a concentration of 5% CO_2 in the nest would indicate a reduction of 25% in the partial pressure of O_2. Due to the unknown sink in the soil a calculation of the O_2 consumption in the nest is not possible. Since the soil might act as a buffer for CO_2, the measured increase in CO_2 concentration might lead to an underestimation of the actual rate of CO_2 production by the colony.

Colony respiration

The cultivation of the fungus by ants and the biochemical interactions between the two symbiotic partners is extremely complex (Schildknecht and Koob 1970; Iwanami 1978). This was the reason why the symbiosis was held intact in the laboratory experiments and the respiration of the whole colony (fungus, workers, and brood) was investigated. Isolated fungus can hardly be maintained without ants, and rapid physiological changes in isolated fungus cannot be excluded.

It should be noted that in our study no experimental separation between the effects of reduced O_2 concentration or increased CO_2 on colony respiration could be made, since any increase in CO_2 concentration is accomplished with a parallel decrease in O_2 concentration. Literature data, however, allow to separately discuss the potential effects of each of the gases on the respiration of workers and fungus. The increased CO_2 concentration should not affect worker respiration or activity, as no immediate physiological effects on insects are known in the range up to 10% CO_2.

In addition, respiratory regulation allows *Atta sexdens* workers to maintain respiratory rates even at hypoxic conditions down to an O_2 concentration of 10% (normoxia 21% O_2) (Hebling *et al*. 1992). Therefore no changes in respiration rates of the ants would be expected in the closed-loop experiment, and observations indicated no noticeable changes in ant activity during the recording period.

A possible effect of increased CO_2 concentrations on fungus growth is the change in acidity of the substrate. Although the pH in the fungus garden was not measured, it is unlikely that acidity due to dissolved CO_2 increased to fungus-inhibiting levels. During the closed-loop experiment the CO_2 concentration was increased only for 3 hours per day, and acidity control by the ants in the fungus garden was assumed (Powell and Stradling 1986).

Based on these arguments, it is suggested that decreased O_2 concentration due to the lack of ventilation immediately leads to suboptimal O_2 supply for the fungus. In closed nests with reduced exchange of respiratory gases, the decrease in the CO_2 respiratory rate is therefore likely to be caused mainly by a reduced respiration of the symbiotic fungus.

Excavation of mature nests revealed a volume of about 2 m³ of fungus chambers (Jonkman 1980). The extrapolation of the measured CO_2 production rate in the closed-loop system leads to an estimation of 140 lCO_2h^{-1} for a mature nest. With such a high production of CO_2 an effective and more or less continuous ventilation of a nest is necessary. This demand stays in conflict, especially in small colonies this demands conflicts with the need of protection against inappropriate weather conditions.

In conclusion, the nest architecture of mature *Atta vollenweideri* colonies ensures a good microclimate for the fungus concerning temperature and humidity. Wind-induced passive ventilation promotes the exchange of respiratory gases and keeps CO_2 concentrations inside the nest at low levels. Small colonies suffer reduced exchange of respiratory gases while closing their nest as a response to heavy rain. As a consequence, the respiration rate of the symbiont fungus is reduced, compromising colony growth. Small colonies therefore are confronted with a tradeoff between water influx and gas exchange in their nests. Whether the reduced number of openings observed in mature nests during winter affects nest ventilation to an extent where gas exchange is adverse to temperature control remains to be shown.