

SHORT COMMUNICATION

Differential construction response to humidity by related species of mound-building termites

Nicole E. Carey^{1,2,*}, Daniel S. Calovi^{1,2,*}, Paul Bardunias³, J. Scott Turner³, Radhika Nagpal^{1,2} and Justin Werfel¹

ABSTRACT

Macrotermes michaelseni and *M. natalensis* are two morphologically similar termite species occupying the same habitat across southern Africa. Both build large mounds and tend mutualistic fungal symbionts for nutrients, but despite these behavioural and physiological similarities, the mound superstructures they create differ markedly. The behavioural differences behind this discrepancy remain elusive, and are the subject of ongoing investigations. Here, we show that the two species demonstrate distinctive building activity in a laboratory-controlled environment consisting of still air with low ambient humidity. In these conditions, *M. michaelseni* transports less soil from a central reservoir, deposits this soil over a smaller area, and creates structures with a smaller volumetric envelope than *M. natalensis*. In high humidity, no such systematic difference is observed. This result suggests a differential behavioural threshold or sensitivity to airborne moisture that may relate to the distinct macro-scale structures observed in the African bushland.

KEY WORDS: *Macrotermes*, Collective construction, Building, Species comparison, Soil transport, Behaviour

INTRODUCTION

The two closely related termite species *Macrotermes michaelseni* and *M. natalensis* coexist sympatrically, are morphologically almost indistinguishable, and demonstrate congruent behavioural characteristics, including the construction of large mounds and the tending and harvesting of fungal symbionts deep inside their nests. Yet despite these similarities, the mound superstructures they create are markedly different. *Macrotermes michaelseni* build tall pillars that can stretch 2 to 3 m high (Fig. 1A) (Turner, 2001), while *M. natalensis* construct short, squat mounds less than 1 m high, without spires (Fig. 1B) (Harris, 1956). It has been hypothesized that some behavioural difference between individual termites of the two species must give rise to this discrepancy (Turner, 2011); however, identification of such has remained elusive (Green et al., 2017). In this paper, we show that although the two species display no significant differences in building activity under still-air conditions with high ambient humidity (>80% relative humidity), when humidity is lowered (<45% relative humidity) significant

differences appear. This suggests some discrepancy in behavioural threshold or sensitivity to airborne moisture, which when extrapolated over a long time scale, may relate to the distinct macro-scale structures observed in the African bushland.

In natural conditions, air humidity is high inside the mound and low outside (Turner, 2001). *Macrotermes* spp., with a permeable cuticle, are vulnerable to desiccation and do not survive long outside the mound (Hu et al., 2012). When exposed to an external environment (and given a suitable substrate), they create tunnels and passageways that shelter them from the harsh, often dry conditions. By confining termites to a laboratory arena and providing them with a soil reservoir with a single restricted access point, we were able to provoke and monitor deposition activity without the potentially confounding effects of excavation (Green et al., 2017).

MATERIALS AND METHODS

Experimental work was conducted at the Cheetah View Field Biology Station near Otjiwarongo, Namibia (20°25'S, 17°4'E). We took groups of 35 major worker termites from three different colonies each of *Macrotermes michaelseni* (Sjöstedt) and *Macrotermes natalensis* (Haviland 1898) and induced them to build on a flat, undifferentiated surface in a humidity-controlled environment (Fig. 1C,D). This environment was created using a feedback loop between three Sensirion SHT85 humidity sensors and an off-the-shelf misting humidifier (UrPower OD-101). Control and sensor recordings were facilitated by an I2C breakout circuit connected to an Intel NUC5i7RYH. The chamber contained a plastic cylinder completely filled with nest soil in its solid Atterberg state (approximately 25% by mass) (ASTM Standard D 4318 test) with an acrylic plate above it. The plate was sanded with a diagonal hatching of fine-grade sandpaper to enable the termites to move easily across the surface.

A 5-mm-diameter circular opening in the centre of the plate gave access to the cylinder of soil underneath, which provided both a source of soil for deposition on the surface and a space where termites could retreat via excavation. We monitored building activity above the surface over 4 h, recording 2D activity (using an RGB camera) and 3D soil deposition (using an infrared depth camera) at 1 Hz (Carey et al., 2017) (Fig. 2, Movie 1). In one set of trials, the ambient humidity inside the chamber was held high (81.3±3.2% relative humidity). A second set held the humidity at 39.9±5.2% relative humidity, close to the natural level of humidity of the outside environment, though not so dry that the termites would desiccate before building had begun. Each of the six colonies provided three groups of termites per treatment, giving $n=9$ trials for each combination of species and humidity condition.

Prior to the experiment, termites were stored in a closed container lined with wet paper towels. Soil was sieved to remove large particulates and all soil cylinders were prepared together, so that

¹Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138, USA. ²School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA. ³Department of Biology, State University of New York College of Environmental Science and Forestry, Syracuse, NY 13210, USA.

*These authors contributed equally to the work

†Author for correspondence (n_carey@g.harvard.edu)

© N.E.C., 0000-0003-1952-5133; D.S.C., 0000-0002-2452-9801

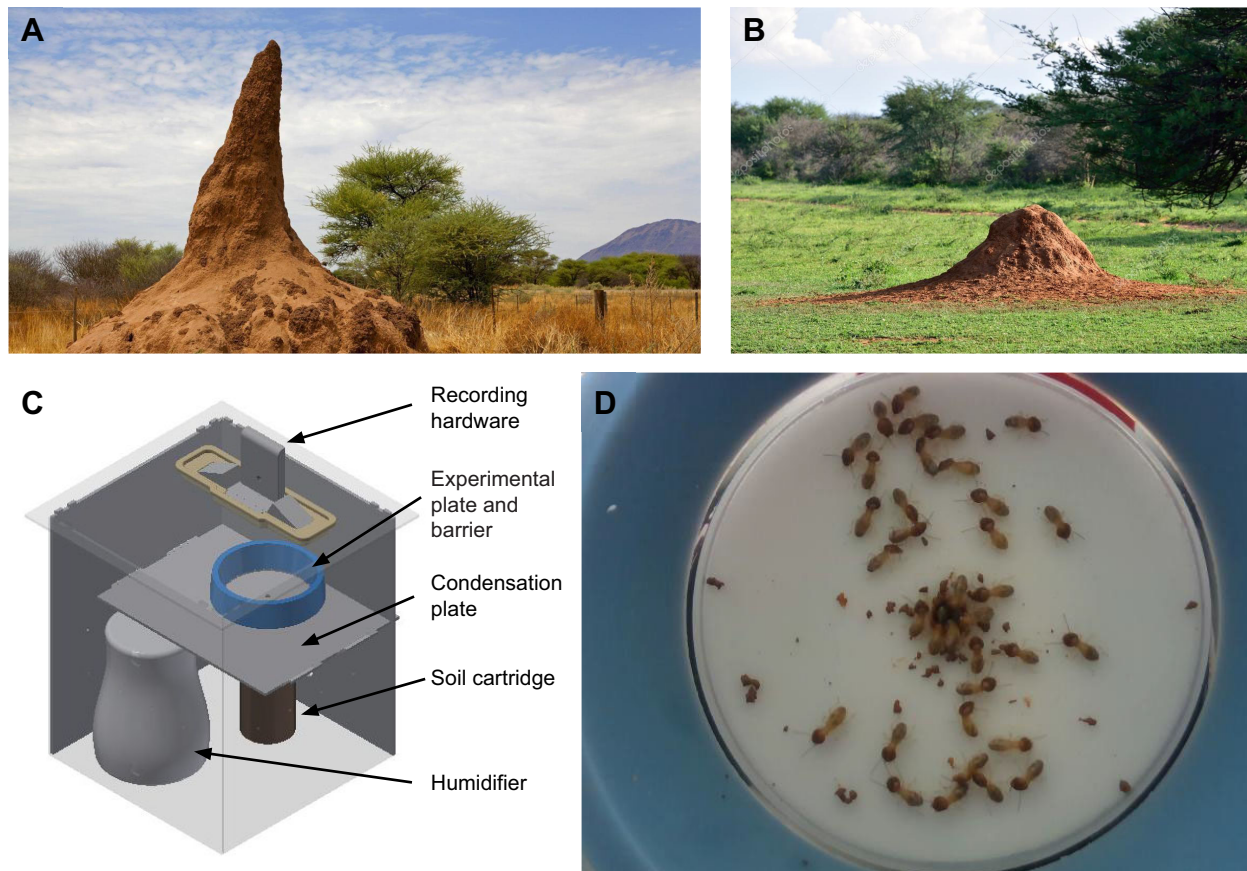


Fig. 1. Experimental subjects. For each experiment, 35 major workers were collected from a colony of either *Macrotermes michaelseni* or *M. natalensis* and encouraged to build in a humidity-controlled environment inside the laboratory. (A) Mound of *M. michaelseni*. (B) Mound of *M. natalensis*. (C) Experimental setup, showing the humidity-controlled environment, recording equipment, build plate for termite construction, and the underlying cartridge acting as a soil reservoir. (D) Termites on experimental plate, viewed from above.

within the same colony, the soil texture and water content was consistent. Condensation from the humidifier was trapped by a condenser plate set just above the humidifier, underneath the build surface, ensuring the arena was not affected by large droplets or water spray (Fig. 1C). A 3D-printed ring structure confined the termites to the area of the build surface directly under the camera. At the end of each experiment, the mud cylinder was detached from the plate, any termites remaining on the plate were removed with forceps, and the resulting build structure was dried completely, then weighed. The area of the plate covered by deposition and the 3D surface map of the resulting build structure (as viewed from above) were also recorded. Custom analysis software used for 3D reconstruction is available from GitHub (<https://github.com/niccarey/RGBD-Environment-Analysis>).

RESULTS AND DISCUSSION

Owing to the low sample size, significance testing was performed using an exact permutation test (<https://www.github.com/lrkrol/permutationTest>; MATLAB, Natick, MA, USA), a non-parametric test that is valid even with an unknown sampling distribution (Hesterberg et al., 2005). In high-humidity conditions, none of the measured parameters registered a statistically significant difference between species. However, in low-humidity (ambient) conditions, the area, volumetric envelope and mass transfer of soil all showed a significant ($P < 0.01$) difference between *M. michaelseni* and *M. natalensis* (Fig. 3). At low humidity, the *M. michaelseni* groups

transferred less soil, covered less of the plate with deposition, and built smaller-volume structures than the *M. natalensis* groups (Fig. 2; Table S2). Previous studies, which have largely taken place at high humidity and did not physically separate excavation from construction (e.g. Green et al., 2017), did not reveal this difference. We note that the density ratio of soil mass to enclosed volume was not significantly different among the different experimental conditions (Table S1), indicating there were no gross differences in the internal structure not visible to our sensors.

These results raise the possibility that sensitivity to air moisture content may drive the differences in large-scale mound morphology, and add an interesting perspective to prior work linking soil and air moisture to the building behaviour of termites and the evolving structure of the mound (Turner, 2011; Soar et al., 2019). The characteristic mound shapes and internal architecture could be an emergent function of the physics of airborne moisture and the termites' own sensory thresholding. If, for example, evaporative flow is constrained to a largely vertical rising water vapour column, vertical building would allow *M. michaelseni* (more sensitive to ambient humidity) to remove the requisite quantities of water-laden soil from the base of the nest without moving outside this environment, leading to tall, narrow mounds; *M. natalensis*, not confined to a central column of high humidity, could build more broadly outward horizontally.

Because both species rely on fungal harvesting for survival, it has been proposed that their mound structures are tuned towards

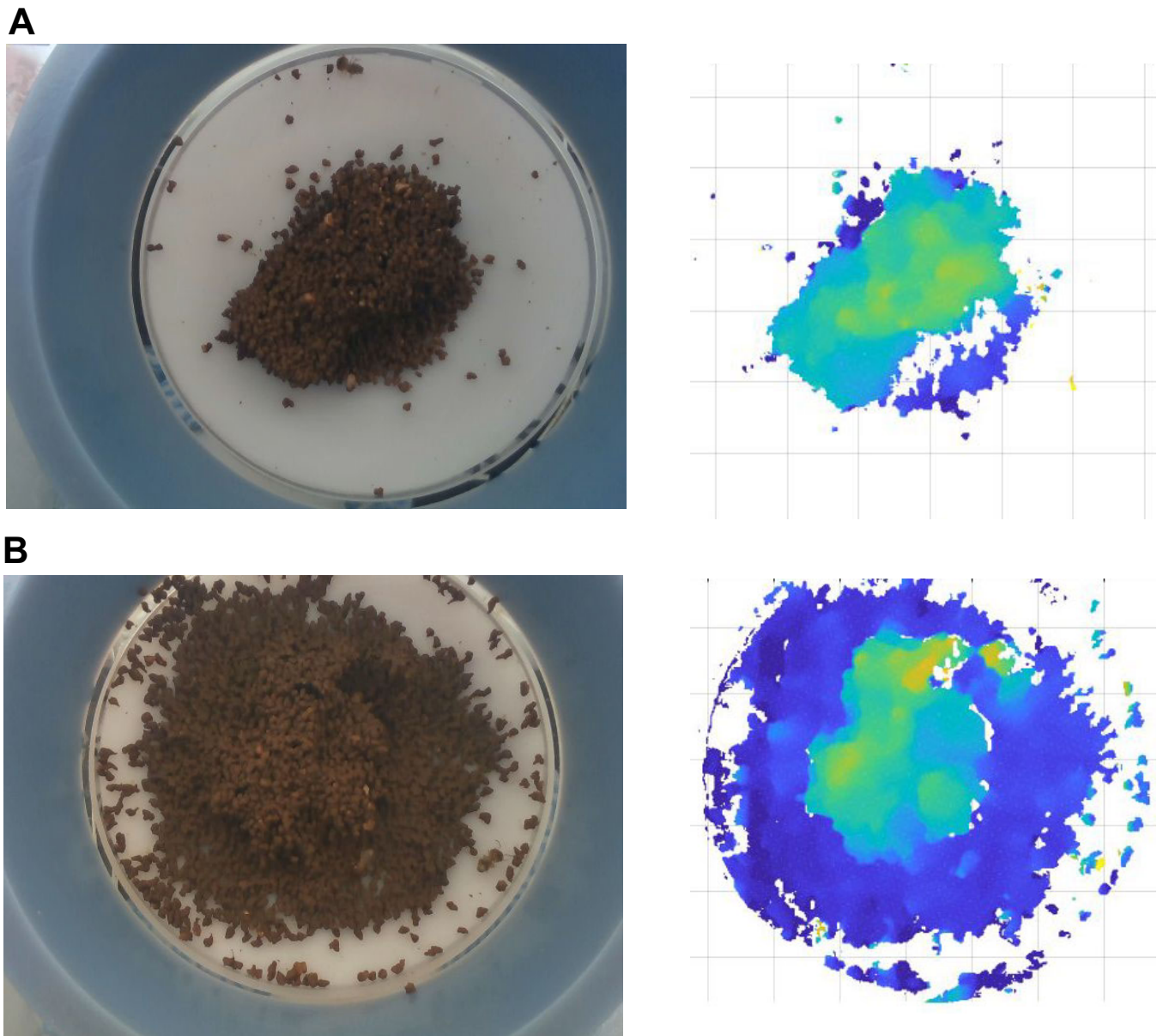


Fig. 2. End-state experimental conditions, viewed from above in RGB and height-coded 3D. (A) Photo (left) and 3D surface reconstruction (right) of typical *M. michaelseni* build under low-humidity conditions. (B) Photo and 3D surface reconstruction of typical *M. natalensis* build under low-humidity conditions.

producing optimal conditions for the health of the fungal colony (Wood and Thomas, 1989). In a similar manner to the observed effect of nest shape on temperature in *M. bellicosus* (Korb and Linsenmair, 1999), the tall towers and large internal pipe structures of *M. michaelseni* mounds could create a different internal humidity setpoint than the squat *M. natalensis* mounds. Whether these different conditions are preferential for cultivation of distinct symbionts is unknown, but *M. natalensis* is associated with a single lineage of *Termitomyces* (Nobre et al., 2011), even though in general fungal diversity in *Macrotermes* is high and likely dependent on horizontal transmission to new colonies (Osiero et al., 2010). Additionally, the fungus combs of *M. michaelseni* produce mushrooms that erupt from the mound to release spores, whereas those of *M. natalensis* do not (Van Der Westhuizen and Eicker, 1991). It has been hypothesized that this is due to contrasting farming methods between *Macrotermes* species (de Fine Licht et al., 2006); however, it is possible, instead, that this discrepancy in the production of fruiting bodies might not

result from direct termite husbandry, but rather from different nest humidity conditions arising from the mound structure. In turn, this could either promote or suppress different strains of *Termitomyces*, or even activate or suppress different stages of the fruiting cycle of a single strain. It is worth noting that *M. michaelseni* and *M. subhyalinus* are known to share *Termitomyces* strands (Osiero et al., 2010; Tilahun et al., 2012), and both build tall mounds (>2 m) with spires or turrets.

It is clear from these experiments that humidity – itself a function of water table seepage, evaporative patterns, fungal metabolism and the actions of the termites themselves – is a key factor likely to influence termites in their role as ecosystem engineers. Another study from our laboratory analyzes the differential response of *M. michaelseni* alone to humidity conditions (P.B., N.E.C., D.S.C., R. Soar, J.S.T., R.N. and J.W., unpublished data). Whether these observed microscale building differences can be directly linked to macroscale structural features of mounds has not yet been

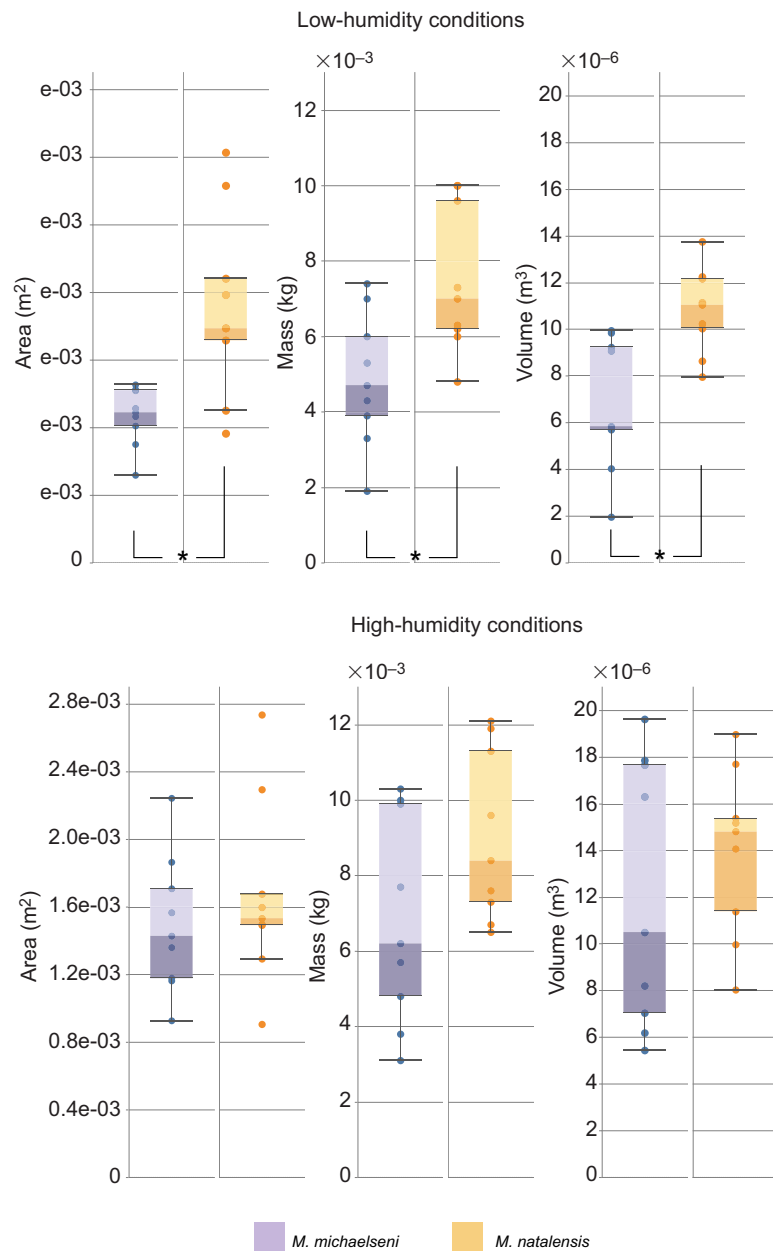


Fig. 3. Comparison of inter-species build parameters under low- and high-humidity conditions. Each box represents a set of $n=9$ experiments; box boundaries show quartiles; whiskers show $1.5\times$ interquartile range. Given the low sample size, we used an exhaustive permutation test to check for differences in means (<https://www.github.com/lrkrol/permutationTest>). Under low-humidity conditions, the data demonstrate significant differences in area ($P=0.001$), mass ($P=0.009$) and volume ($P=0.003$) between the two species. Under high-humidity conditions, our tests revealed no differences below threshold significance ($P>0.05$ for all metrics; Table S1).

demonstrated, but these experiments represent a promising direction for further investigation. We note in particular that other ‘pairs’ of related termite species occupying the same geography but building dissimilar mounds have been observed on multiple continents (Darlington, 1997; Araújo et al., 2017; Coles de Negret and Redford, 1982); investigating behavioural thresholding between these species may yet give us more insights into the design and function of mound morphology.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.E.C., D.S.S., P.M.B., J.T., R.N., J.W.; Methodology: N.E.C., D.S.S., P.M.B., J.W.; Software: N.E.C.; Formal analysis: N.E.C., D.S.S.;

Investigation: N.E.C., D.S.S.; Writing - original draft: N.E.C.; Writing - review & editing: D.S.S., P.M.B., J.W.; Visualization: N.E.C.; Supervision: J.T., R.N., J.W.; Project administration: J.W.; Funding acquisition: J.T., R.N., J.W.

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Supplementary information

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References

Araújo, A. P. A., Cristaldo, P. F., Florencio, D. F., Araújo, F. S. and DeSouza, O. (2017). Resource suitability modulating spatial co-occurrence of soil-forager termites (Blattodea: Termitoidea). *Aust. Entomol.* **56**, 235-243. doi:10.1111/aen.12226

- Carey, N., Nagpal, R. and Werfel, J. (2017). Fast, accurate, small-scale 3D scene capture using a low-cost depth sensor. In 2017 IEEE Winter Conference on Applications of Computer Vision (WACV), pp. 1268-1276. IEEE.
- Coles de Negret, H. R. and Redford, K. H. (1982). The biology of nine termite species (Isoptera: Termitidae) from the cerrado of central Brazil. *Psyche A J. Entomol.* **89**:81-106. doi:10.1155/1982/36726
- Darlington, J. P. E. C. (1997). Comparison of nest structure and caste parameters of sympatric species of *Odontotermes* (Termitidae, Macrotermitinae) in Kenya. *Insectes Soc.* **44**, 393-408. doi:10.1007/s000400050060
- de Fine Licht, H. H., Boomsma, J. J. and Aanen, D. K. (2006). Presumptive horizontal symbiont transmission in the fungus-growing termite *Macrotermes natalensis*. *Mol. Ecol.* **15**, 3131-3138. doi:10.1111/j.1365-294X.2006.03008.x
- Green, B., Bardunias, P., Turner, J. S., Nagpal, R. and Werfel, J. (2017). Excavation and aggregation as organizing factors in de novo construction by mound-building termites. *Proc. R. Soc. B Biol. Sci.* **284**, 20162730. doi:10.1098/rspb.2016.2730
- Harris, W. V. (1956). Termite mound building. *Insectes Soc.* **3**, 261-268. doi:10.1007/BF02224306
- Hesterberg, T., Moore, D. S., Monaghan, S., Clipson, A. and Epstein, R. (2005). Bootstrap methods and permutation tests. *Introduct. Practice Stat.* **5**, 1-70.
- Hu, J., Neoh, K.-B., Appel, A. G. and Lee, C.-Y. (2012). Subterranean termite open-air foraging and tolerance to desiccation: Comparative water relation of two sympatric *Macrotermes* spp. (Blattodea: Termitidae). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **161**, 201-207. doi:10.1016/j.cbpa.2011.10.028
- Korb, J. and Linsenmair, K. E. (1999). The architecture of termite mounds: a result of a trade-off between thermoregulation and gas exchange? *Behav. Ecol.* **10**, 312-316. doi:10.1093/beheco/10.3.312
- Nobre, T., Koné, N. A., Konaté, S., Linsenmair, K. E. and Aanen, D. (2011). Dating the fungus-growing termites' mutualism shows a mixture between ancient codiversification and recent symbiont dispersal across divergent hosts. *Mol. Ecol.* **20**, 2619-2627. doi:10.1111/j.1365-294X.2011.05090.x
- Osiemo, Z. B., Marten, A., Kaib, M., Gitonga, L. M., Boga, H. I. and Brandl, R. (2010). Open relationships in the castles of clay: high diversity and low host specificity of *Termitomyces* fungi associated with fungus-growing termites in Africa. *Insectes Soc.* **57**, 351-363. doi:10.1007/s00040-010-0092-3
- Soar, R., Amador, G., Bardunias, P. and Turner, J. S. (2019). Moisture gradients form a vapor cycle within the viscous boundary layer as an organizing principle to worker termites. *Insectes Soc.* **66**, 193-209. doi:10.1007/s00040-018-0673-0
- Tilahun, A., Kebede, F., Yamoah, C., Erens, H., Mujinya, B., Verdoodt, A. and Van Ranst, E. (2012). Quantifying the masses of *Macrotermes subhyalinus* mounds and evaluating their use as a soil amendment. *Agric. Ecosyst. Environ.* **157**, 54-59. doi:10.1016/j.agee.2011.11.013
- Turner, J. S. (2001). On the mound of *Macrotermes michaelseni* as an organ of respiratory gas exchange. *Physiol. Biochem. Zool.* **74**, 798-822. doi:10.1086/323990
- Turner, J. S. (2011). Termites as models of swarm cognition. *Swarm Intell.* **5**, 19-43. doi:10.1007/s11721-010-0049-1
- Van Der Westhuizen, G. C. A. and Eicker, A. (1991). The 'omajowa' or 'termitenpilz', *Termitomyces* sp. (Agaricales) of Namibia. *South Afr. J. Bot.* **57**, 67-70. doi:10.1016/S0254-6299(16)30986-3
- Wood, T. and Thomas, R. (1989). The mutualistic association between Macrotermitinae and *Termitomyces*. In *Insect-Fungus Interactions* (ed. N. Wilding, N. M. Collins, P. M. Hammond and J. F. Webber), pp. 69-92. New York: Academic Press.