ESM: The effects of disturbance threat on leaf-cutting ant colonies: a laboratory study

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Fig. S1 Mean ± s.e A) proportion of positive mandible opening responses (MORs) to four different threat stimuli for all 260 ants from both long term (17 month) disturbed and undisturbed colonies, B) length of MOR to four different threat stimuli for all ants . The latter treatment was designed to simulate a vertebrate predation threat, which should be a specific stimulus for defensive workers to respond to. Different letters above columns indicate significant differences between MOR stimuli at $P < 0.05$ based on pairwise comparisons following GLMM analysis with P values adjusted using the sequential Bonferonni method.

Fig. S2 Mean ± s.e A) proportion of positive mandible opening responses (MORs) to two different threat stimuli for all 252 ants from both short term (3 week) threat disturbed and control sub-colonies, and B) proportion of positive mandible opening responses (MORs) to

two different stimuli for 252 ants from both threat disturbed and control sub-colonies two weeks after the end of the short term disturbance treatment. Asterisks indicate significant differences between MOR stimuli (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

Fig. S3 Mean ± s.e. sizes (ml of fungus garden) over a 17 month period of leaf-cutting ant colonies that were either disturbed or undisturbed. The sizes of disturbed and undisturbed colonies did not differ significantly over the course of the experiment (repeated measures GLM: $F_{1,131} = 0.880$, $P = 0.350$)

Fig S4 Mean ± s.e counts of large workers observed during a 17 month period where leaf-cutting ant colonies were either disturbed or undisturbed once colonies started to produce larger workers. The number of large workers produced over this time period did not differ significantly between disturbed and undisturbed colonies (repeated measures GLM $F_{1,79} = 0.596$; $P = 0.442$).

Fig S5 Schematic of how leaf-cutting ant colonies were housed in the laboratory. Fungus gardens were housed in clear plastic boxes, covered with a flower pot lid to keep the fungus humid and in the dark. Both the transparent box and the flower pot had a small hole at the base to allow ants to move freely between the nest environments and outside. These were then placed inside a larger box coated with fluon. Inside the box, water (in a falcon tube plugged with cotton wool) and leaves (placed in a foraging box as illustrated by the green rectangle) were available *ad libitum*.

Fig. S1

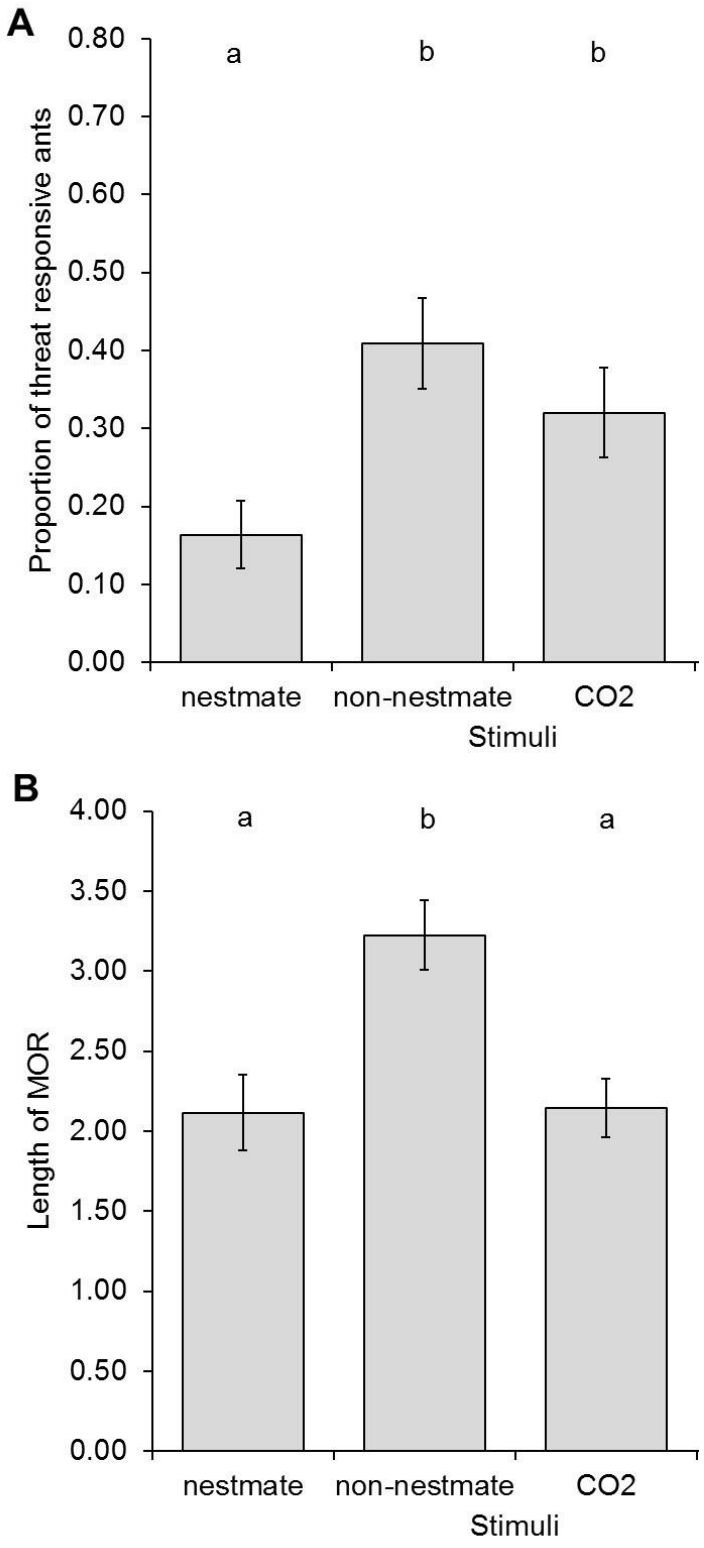
A
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Proportion of threat responsive ants
a
b
b
nestmate
non-nestmate
CO2
Stimuli
B
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Length of MOR
a
b
a
nestmate
non-nestmate
CO2
Stimuli

Fig. S2

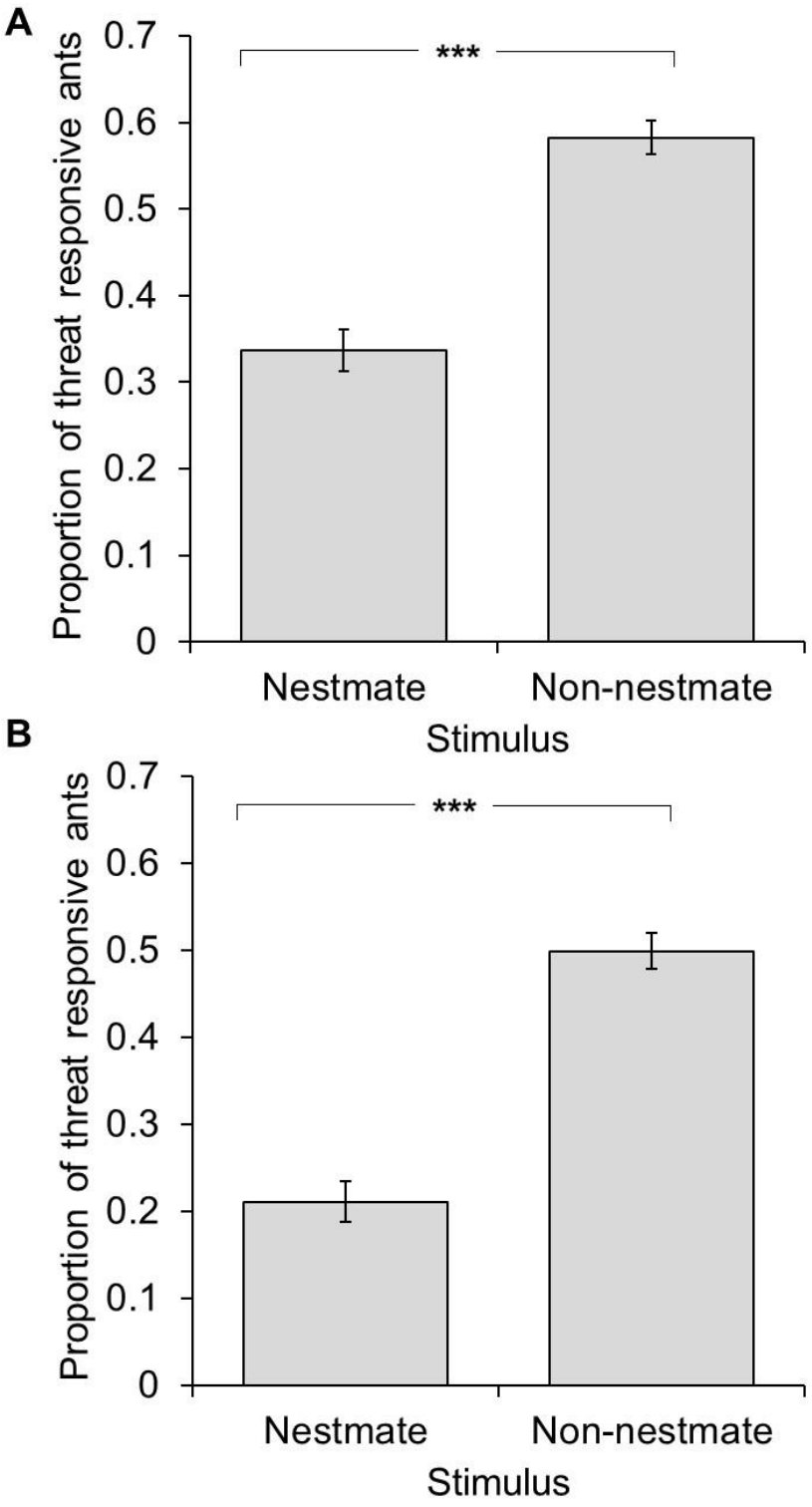
A
Proportion of threat responsive ants
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
Nestmate
Non-nestmate
Stimulus
B
Proportion of threat responsive ants
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
Nestmate
Non-nestmate
Stimulus

Fig. S3

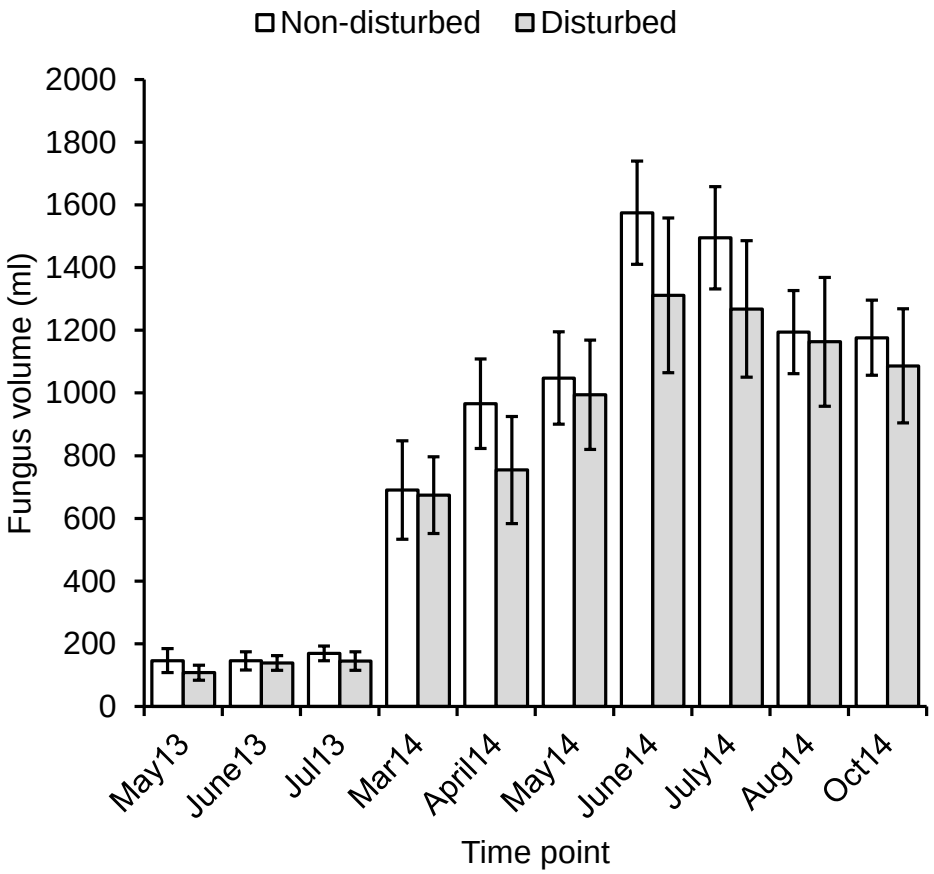
Non-disturbed
Disturbed
Fungus volume (ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
May13
June13
Jul13
Mar14
April14
May14
June14
July14
Aug14
Oct14
Time point

Fig. S4

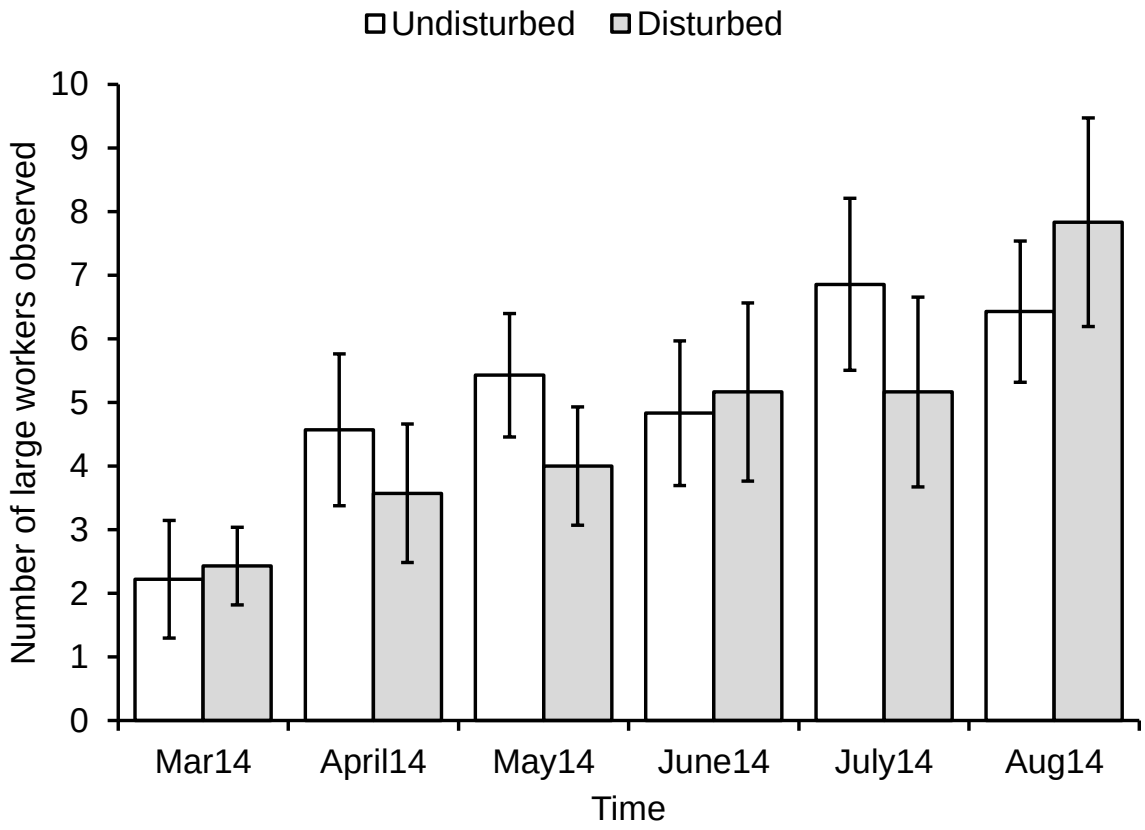
Undisturbed
Disturbed
Number of large workers observed
0
1
2
3
4
5
6
7
8
9
10
Mar14
April14
May14
June14
July14
Aug14
Time

Fig. S5