1) In the metabolic model, I assume all growth functions, such as RNA, and protein production are included? Does the model assume that these other functions, such as protein production etc, happen at similar rates during the day as during the night?

Yes, production of RNA and protein are also included. The rate of production is related to the intensity of light, so that these rates are different during the day and night.

2) The organisms were cultured at 4.5-10 umol m-2 s-1 (L83). How does this compare to the desert light levels, and how would an increase in light level affect the conclusion of this paper regarding wet/dry cycles during day/night? Is the light level PAR or total radiation?

While the radiation incident on the crust surface can be orders of magnitude higher that the one we used, it is subject to intense multiple scattering losses, so that only 1 percent of incident radiation remains a couple of millimeters down into the soil. *M. vaginatus* makes a living within this steep light gradient, usually in the subsurface, coming up to the surface only when light intensity is very moderate, (morning, overcast conditions) and it has a "shade plant" phenotype (low photosynthesis saturation intensity, heavy complement of light harvesting pigments and no sunscreen pigments). Isolate *M. vaginatus* does not grow in liquid media under desert light levels that we used previously to mimic more natural conditions in the lab with intact biocrusts (~600 umol m-2 s-1; DOI: 10.1038/ismej.2013.83).
We conducted our experiments close to the growth optimum of *M. vaginatus* in liquid media. The light level given is PAR.

This text has been added to the manuscript in the methods section (section 2.2)

Related topic, in the results and discussion (L 198, 199; Table 3) two light levels are defined. It was not clear when reading the paper what this meant, whether this was caused by a change in biomass production or changed light levels. Please clarify.

Thanks for pointing this out. The manuscript has been changed to clarify this:
L83: Because different culture containers were used between respiration experiments and biopolymer experiments, they possessed different photon fluxes (measured in $\mu mol\ g^{-1}\ h^{-1}$), which is reflected in Table 3. This is accounted for in simulations.

3) L 116: please clarify why crotonic acid was determined. As far as I know, it is not mentioned in the results and discussion but seems to be the breakdown product of polyhydroxybutyrate. (?)

The manuscript has been changed to clarify this:

Section 2.6 Title: "Quantification of PHB via LC/MS Measurement of Crotonic Acid"
L117: "Polyhydroxybutyrate breaks down to form crotonic acid in strongly acidic environments. The quantity of crotonic acid formed is used to calculate PHB quantity."

4) As a clarification, please explain why biopolymer reactions are included in the model, but not found in the genome. Similar for the other processes.

These clarifications have been added to the manuscript:

L133: "Automatic annotations were further refined through manual annotation. This was necessary because automated modelling databases occasionally do not contain strong homologs for a certain function, and thus fail to assign it to the genome via simple homology search algorithms."

L144: "...reactions for the synthesis and metabolism of biopolymers polyphosphate, glycogen and PHB, which were not given within the automated annotation, were added after being found in the genome through extensive manual annotation."

L165: "Automated model generation typically does not assume that biopolymers act as resources that may accumulate or deplete."

5) L 150: how were LB and UB determined. On the one hand they seem to be the product of the model (Table 3) but at the same time constrain the model (L 150).

In Table 3 measured UB and LB refer to the experimental bounds, i.e. the standard deviation. Modelled UB and LB refer to the upper and lower values obtained from sensitivity analysis. In modelled constraint reactions for light, UB and LB represent the assumed deviation that is input into sensitivity analysis.

The reaction flux UB and LB used as inputs in the model are fixed at the experimentally measured values (see L176, eqn 6). In sensitivity analysis, this is varied over the estimated deviation (See L182-186).

Due to this confusion, the LB and UB in Table 3 has been amended as seen below.

"Table 1: Experimental and modelled flux values over light and dark conditions. Constraint fluxes are noted with a "*". Negative and positive CO_2 fluxes represent uptake and respiration respectively, while negative and positive biopolymer flux rates represent depletion and accumulation respectively. Measured "-" and "+" refer to the standard deviation. Modeled "-" and "+" refer to the upper and lower values obtained from sensitivity analysis. In modeled constraint reactions for light, "-" and "+" represent the assumed deviation that is input into sensitivity analysis."

	Measured			Modeled		
	Flux ($\mu mol\ g^{-1}\ h^{-1}$)	-	+	Flux ($\mu mol\ g^{-1}\ h^{-1}$)	-	+

Light (1)						
Light*	141			141	93	211
CO_2	-19.6	-23.3	-15.9	-33.4	-50.3	-21.8
Light (2)						
Light*	4670			4670	3551	6290
Glycogen	9.99	9.47	10.51	3.07	0.00	9.30
PHB	0.0366	0.0050	0.0683	0.121	0.044	0.250
Polyphosphate	2.28	1.57	2.99	2.82	1.00	5.70
Dark						
Light*	0.00			0.00		
Glycogen*	-2.49	-6.08	1.09	-2.49	-9.66	4.67
PHB*	-0.14	-0.17	-0.11	-0.136	-0.20	-0.07
Polyphosphate*	-0.02	-1.27	1.22	-0.02	-2.51	2.46
CO_2	17.3	14.1	20.6	11.9	0	48.5

6) Table 1: what is the relevance of the reactions mentioned such as hydrogen production in the table, but neither curated not found in the genome.

Fermentation and nitrogen fixation are known to be important pathways in biocrust metabolism. We annotated these to investigate if *M. vaginatus* could play a key role. Hydrogen production was annotated because hydrogen evolution was measured from crusts in previous experiments, though not reported.

7) L 170-173 and Table 2: I am not familiar with flux balance calculations, so the phrase PHB -> nothing is confusing? Please add explanation in one additional sentence.

This has been changed:

L167: "…where A is a side reactant, B is a side product, X is a polymer subunit, and X_n is a polymer with length n. 'Nothing" is not a physical term, but a mathematical way to describe resource accumulation in a steady state simulation."

8) Table 3 and Fig. 2c seem to have some overlap.

This is true, they are just different representations of the same data, we've decided it best to keep them because they may be useful to different types of readers. While the graph provides a visual comparison of experiment and model predictions, the tabulation of values may be helpful for those performing future simulations and studies.

9) L 214: please elaborate how polyP can be used in other ways than an energy source

This has been corrected as described below:

L213-214: "Polyphosphate is likely an important biopolymer for *M. vaginatus* across many different stressed conditions as a reservoir of phosphate for later growth, though so-called "luxury uptake" and storage when growth is halted by some other factor, and as a reservoir of energy in the form of phosphate-phosphate bonds under conditions of abundant energy generation, phosphate and a lack of conditions to use it for growth or homeostasis. This importance of polyphosphate has been identified in gene expression studies (Rajeev et al., 2013)."

10) L 218: change consistent to constant or words similar to that.

Done

L218: "…storage polymer or for other metabolic activities that do not require a constant energy source, such as replication."

L 52: diel does not need to be capitalized

Done

L52: "During the diel cycle…"

L 63: the use of the phrases dark and light reactions are confusing: they have very specific meaning in the study of photosynthesis, but I don't think that is what is meant here. Please Âˇn replace with something like metabolism in the dark versus in the light.

Good point. We avoid those terms now in the manuscript to avoid confusion.

L63: "Therefore, we expect wet-up and dry-down metabolism in the dark likely have fixed biopolymer costs whereas metabolism in the light enables replenishment of biopolymer reserves."

L71: complex sentence that can be simplified.
Done

L70-71: “We interpret these results using a simple cost/benefits framework. The “cost” is biopolymer depletion in the dark, and the “benefit” is biopolymer accumulation in the light.

L 94: add rcf to the list of abbreviations, and add units
Done
Changed to “x g” (g-force). All units will be added to the abbreviations.

L 134: what are GPR relations

The Gene-Protein-Reaction relation. It is a standardized description of the link between a gene, its associated protein, and the associated reaction in genome-scale metabolic models. We will include this in abbreviations.

L 135: comma after databases can be removed
Done
L135: “…analysis, and the databases KEGG and MetaCyc…”

L 168: why the word “side” with reactant and product?

The reactants and products of interest are the biopolymer and its subunit. A and B are used to generalize other chemicals involved in the process.

L 194: change profiles to concentrations
Done
L194: “…carbon dioxide profiles concentrations varied linearly with time…”

L 246: Add year after reference (Knoop)
Done
L246: “Knoop *et al.* (2013)”

L 247: is the efficiency measured at the same light level?

No, the rates are normalized to the photon intensity in this comparison.