

## Review of: "Pollen meta-barcoding reveals foraging preferences of honeybees (Apis mellifera L.) along space-time gradient in Japan"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Ref., Pollen meta-barcoding reveals foraging preferences of honeybees (Apis mellifera L.) along space-time gradient in Japan

The paper covers very important and up-to-date subject concerning the available food recources for Apis mellifera, the main pollinator of crop plants worldwide, in rural-urban gradient. Shortages of resources or diet inadequate in quality are indicated among a key drivers responsible for decline in bees colonies. Therefore, the presented study is fully justified.

In general, I found the study good designed and clearly presented. Methods are sufficiently described. The landscape types were sufficiently defined (based on the diversity, connectivity, and aggregation of the patches). Molecular analysis were employed for the pollen identification, which is a new method employed for pollen resources identification (more advanced than botanical taxa identification using light microscope).

However, I can not agree with some conclusions presented in the Discussion section.

L300-400 – The statement "This means that these grass species could be implemented in rural and urban management recommendations".

It is known from many studies that grass pollen (anemophilous plants) are present in bee products (honey, pollen loads, bee bred). However, we can not interpret this as honeybee preference towards grass species. It is more possible that grass pollen contaminate the samples and can be overrepresented due to high amount of produced pollen grains (amount of anemophilous species vs insect pollinated species; important isn't it ???). Moreover, we should treat the data of grass pollen representation in sample as various direct observations (honeybee activity on flowers) did not confirm that honey bees use their pollen. This pollen can be used in the periods of hanger related to lack of other sources availability. The other thing is a quality of grass pollen (e.g. low protein content)

Therefore, I suggest to change your statement and do not recommend grasses for management -makers for improvement of food resources for pollinators. There are much more preferable plant species with more adequate for establishment of bees balanced diet (in amount and quality.

L 415-417. I agree that Trifolium is very important food source for bee diet due to balanced macro- and microelements. For details see e.g., Filipiak et al., 2017. Ecological stoichiometry of the honeybee: Pollen



diversity and adequate species composition are needed to mitigate limitations imposed on the growth and development of bees by pollen quality. Plos One  $\underline{10.1371/journal.pone.0183236}$ 

Minor remarks

Refeerences

Spelling should be checked and corrected.

I found a lot of typo errors, e.g. No 46 Steffan-dewenter – should be Steffan-Dewenter;

No 54 - apis mellifera; should be Apis mellifera

No 56 mapview - should be Map....

N 60 raster- should be Raster

No 74 - Please change capital letters to small in authors names

No 86 parvise..... Change for Parvise

Please, check carefully all References

Table 1 -

2<sup>nd</sup> column - Prefectu??? correct

last column should be Precipitation