**Morau & Piepho:** **Interactions between abiotic factors and the bioactivity of biodynamic horn manure on the growth of garden cress (*Lepidium sativum* L.) in a bioassay**

**Additional file**

**Contents**

**1- Description of supplemental statistical analyses 1**

1.1 Model 2: Integrating the factor *day* 2

1.2 Model 3: Integrating the factor *position* 3

1.3 Model 4: Analysis of one hanger 3

1.4 More liberal comparison method for Model 1 4

**2. Additional Tables S1-S7 5**

**3. Additional Figures S1-S3 11**

**1- Description of supplemental statistical analyses**

**1.1. Model 2: Integrating the factor *day***

In Model (1), the traits at different days are analysed individually. An overall analysis of the growth was performed by integrating the factor *day* as follows (Model 2):

Y mijkl = µ + bi + fj + bfij + dm + dbmi + dfmj + dbfmij + tk + dtmk + dftmjk + dftwmjkl + emijkl

Where:

* µ, bi, fj, bfij, and tk as defined for Model (1)
* dm is the fixed effect of the m-th *day*
* dbmi is the fixed effect of the i-th level of the *dose* factor at m-th *day*
* dfmj is the fixed effect of the j-th level of the *test* factor at m-th *day*
* dbfmij is the fixed effect of the interaction between the i-th level of the *dose* factor and the j-th level of the *test* factor at m-th *day*
* dtmk is the random effect of the k-th trial at m-th *day*
* dftmjk is the random effect of the jk-th hanger at m-th *day*
* dftwmjkl is the random effect of the l-th block in the jk-th hanger at m-th *day*
* emijkl is the random effect of the ijkl-th bag at m-th *day*
* Ymijkl is the mean of root or hypocotyl length in the ijkl-th bag at m-th *day*

In case of variance heterogeneity for *day,* a log-transformation was performed. For each series, p-values (Wald F-test) of Model (2) are presented in Table 2.

For the individual analysis for each trait, Model (1) was preferred because of the possibility to fit specific variances for the test factors and the avoidance of a logarithmic transformation.

**1.2 Model 3: Integrating the factor *position***

To analyse the influence of the seedling position in the bag, it was required to define one observation as one seedling instead of as one bag in Model 1. This analyse was performed separately for each factor level of each Series with the following Model (3):

 Yijkl = µ + bi + pj + pbij + tk + btik + twkl + btwikl + eijkl (3)

Where:

* µ, bi, and tk as defined for Model (1)
* pj is the fixed effect of the jth-position (j = 1-16)
* pbij the fixed effect of the interaction between the j-th position and the i-th treatment
* btik is the random effect of the interaction between the i-th *dose* treatment and the k-th trial
* twkl the random effect of the l-th block of the k-th trial
* btwikl the random effect of the ikl-th bag
* eijkl theerror effect of the ijkl-th seedling
* Yijkl thelength of the ijkl-th seedling

The results are documented in the Supplemental Figures S1, S2 and S3.

**1.3 Model 4: Analysis of one hanger**

To evaluate the reproducibility of the bioassay, each replication (i.e. one single hanger) was independently analysed according to a randomized complete block design (n = 20). The analysis was performed for root length at day 7 (Series W and G) and day 8 (Series L) with the following linear mixed Model (4):

Yijk = µ + bi + wj + bwij + eijk (4)

Where:

* µ the overall effect
* bi thefixed effect of the i-th treatment (Series W and G: i=1-3; series L: i=1-2)
* wj the random effect of the j-th block (j=1-20)
* bwij the random effect of the ij-th bag
* eijk the random effect of the k-th seedling (k=1-16) in the ij-th bag
* Yijk the root or hypocotyl length of the k-th seedling in the ij-th bag

The results are documented in the Supplemental Table S5.

**1.4 More liberal comparison method for Model 1**

In the comparison approach performed for Model (1) in the main text, the treatment means were compared with the LSMEANS statement, using the Tukey-Kramer-test to control the family-wise Type I error rate. This approach is conservative because it also accounts for comparisons of treatments differing in both *dose* and *volume*.

Another, more liberal comparison approach, which compares *doses* only at the same level of *volume*, and vice versa, can be conducted as follows. The *dose* treatment means at the same *volume* level were compared with the SLICE statement. The *volume* treatment means at the same *dose* control (Variant: Control)were compared the same way. To control the family-wise Type I error rate, these comparisons were adjusted with the Tukey-Kramer procedure. Furthermore the significance level α was adjusted to 0.05 / *a,* with *a* the number of slices. This corresponds to a Bonferroni correction.

The results of this alternative approach, which produced a few more significant results than the conservative approach, are documented in the Supplemental Tables S1, S2 and S3.

**2. Additional Tables and Figures**

**Table S1.** **Average lengths (mm) for all growth traits in series W.** Means (mm) and standard error (s.e; by variance heterogeneity: minimum and maximum) are detailed. The pairwise comparisons were conducted with the alternative, more liberal method described in Section 1.4. At a time point (in column), *dose* treatments by the same *volume* level with no lowercase in common differ significantly (in bold, *p* < 0.0167, Tukey-Kramer-test by the same *volume* level); *volume* treatments by the *dose* level ‘Control’ with no uppercase in common differ significantly (*p* < 0.0167, Tukey-Kramer-test by the same *dose* level).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Volume | Dose | Hypocotyl length (mm) |  | Root length (mm) |
|  |  | day 3 | day 4 | day 5 | day 6 |  | day 2 | day 3 | day 4 | day 5 | day 6 | day 7 |
| 4 ml | D0.1µl | 17.48  | 35.93  | 49.67  | 59.86 |  | 14.77 | 39.28 | 67.35 | 83.40  | 91.93  | (91.93) 1  |
|  | D1µl | 17.45 | 35.91  | 49.90  | 60.30 |  | 14.77 | 39.21 | 67.48 | 83.97  | 92.80  | (92.80) 1  |
|  | C | 17.30 B | 35.84 | 49.60 B | 59.83 C |  | 14.66 | 38.90 A | 67.88 A | 84.96 A | 93.52 A  | (93.52 A) 1  |
| 5 ml | D0.1µl | 17.49 | 35.62  | 50.70 | 61.96 |  | 14.02 | 35.86 | 58.07 | 70.13 | 79.02 | **84.83** a |
|  | D1µl | 17.30 | 35.80 | 51.07 | 62.44 |  | 13.92 | 35.34 | 57.06 | 69.26 | 78.92 | **85.04** a |
|  | C | 17.34 B | 35.77 | 50.88 B | 62.02 B |  | 13.93 | 36.00 B | 58.43 B | 69.87 B | 76.84 B | **81.34** B b |
| 6 ml | D0.1µl | 17.83 ab | 36.17 ab | 52.22 ab | 63.96 |  | 14.05 | 34.57 | 52.49  | 63.48 | 71.78 | 77.69 |
|  | D1µl | **17.68 b** | **35.72 b** | **51.85 b** | 63.79 |  | 14.04 | 34.34 | 51.82  | 63.13 | 72.67 | 79.84 |
|  | C | **18.20 A a** | **36.81 a** | **52.74 A a** | 64.35 A |  | 14.22 | 35.04 B | 53.59 B  | 63.96 B | 70.92 B | 76.37 B |
| s.e. |  | 0.45 | 1.33 | 1.23 | 1.20 |  | 0.54 | 1.62 | 2.18 | 2.60-2.67 | 2.81-2.94 | 2.79-2.95 |

1 For variant 4ml, root length at day 7 was not measured. Root length at day 6 was used to perform the final statistical analysis at the end of experiment.

**Table S2.** **Average lengths (mm) for all growth traits in series G.** Means (mm) and standard error (s.e.) are detailed. The pairwise comparisons were conducted with the alternative, more liberal method described in Section 1.4. At a time point (in column), *dose* treatments by the same *laying time* level with no lowercase in common differ significantly (in bold, *p* < 0.0125, Tukey-Kramer-test by the same *laying time* level); *laying time* treatments by the *dose* level ‘Control’ with no uppercase in common differ significantly (*p* < 0.0167, Tukey-Kramer-test by the same *dose* level).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Layingtime | Dose | Hypocotyl length (mm) |  | Root length (mm) |
|  |  | day 3 | day 4 | day 5 | day 6 |  | day 2 | day 3 | day 4 | day 5 | day 6 | day 7 |
| 0min | D0.1µl | 17.37  | 34.72  | 51.38  | 62.69  |  | 14.58 | **35.62** b | 57.25  | 69.72  | 78.02 | 84.75 |
|  | D1µl | 17.54  | 34.99  | 51.80  | 63.04 |  | 14.63 | 35.81 ab | 57.38  | 69.80 | 77.91 | 84.86 |
|  | C | 17.69 AB | 35.30 AB | 51.90 AB | 63.10 B |  | 14.79 | **36.52** A a | 59.10 A  | 72.12 A | 80.12 A | 86.53 A |
| 20min | D0.1µl | 19.22 | 37.89 | 54.48 | 65.34  |  | 15.06 | 32.81  | 47.77  | 58.03 | 66.29 | 73.29 |
|  | D1µl | 19.10 | 37.66 | 54.31 | 65.16 |  | 15.07 | 32.59  | 47.25 | 57.48 | 65.88  | 73.44  |
|  | C | 19.33 A | 38.08 A | 54.68 A | 65.41 A |  | 15.06 | 32.99 AB | 48.24 B | 58.31 B | 65.71 B | 71.73 B |
| 40min | D0.1µl | 16.14 | 33.97 | 51.57  | 63.31 |  | 14.18 | 29.08  | 45.79 | 58.30  | **67.77** a | **74.94** a |
|  | D1µl | 16.10 | 33.84 | 51.42  | 63.32 |  | 14.22 | 28.88 | 45.07 | 57.67  | **67.87 a** | **75.87** a |
|  | C | 15.99 B | 33.50 B | 50.96 B  | 62.89 B |  | 14.23 | 28.67 C | 44.62 B | 55.65 B | **63.58** B b | **69.32** B b |
| 60min | D0.1µl | 16.56  | 34.22 | 51.21 | 62.39  |  | 14.48 | 29.61 | 45.12 | 56.98 | 65.95 | 72.80  |
|  | D1µl | 16.40 | 34.10 | 51.42 | 62.85  |  | 14.43 | 29.08 | 44.20  | 55.99 | 65.86 | 74.26 |
|  | C | 16.62 B | 34.41 B | 51.63 B | 62.90 B  |  | 14.41 | 29.37 BC | 44.92 B | 56.39 B | 64.42 B | 70.25 B  |
| s.e. |  | 0.71 | 1.07 | 0.78 | 0.58 |  | 0.28 | 1.21 | 1.75 | 1.92 | 1.87 | 1.80 |

**Table S3.** **Average lengths (mm) for all growth traits in series L.** Means (mm) and standard error (s.e.) are detailed. The pairwise comparisons were conducted with the alternative, more liberal method described in Section 1.4. At a time point (in column), *dose* treatments by the same *light* level with no lowercase in common differ significantly (in bold, *p* < 0.01, Tukey-Kramer-test by the same *light* level); *light* treatments by the *dose* level ‘Control’ with no uppercase in common differ significantly (*p* < 0.025, Tukey-Kramer-test by the same *dose* level).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Light | Dose | Hypocotyl length (mm) |  | Root length (mm) |
|  |  | day 3 | day 4 | day 5 | day 6 |  | day 2 | day 3 | day 4 | day 5 | day 6 | day 7 | day 8 |
| FL 100  | D1µl | 17.11  | 34.11  | 51.94 | 63.16 |  | 13.48 | 30.91 | 47.13  | 59.38  | 68.37  | 75.59  | 80.51  |
|  | C | 17.12 A | 33.96 AB | 51.73 | 63.06 |  | 13.56 | 31.12 A | 46.78 A | 57.66 A | 64.90 A  | 70.96 A  | 75.03 A  |
| FL 500 | D1µl | 16.52  | 33.75  | 51.31 | 62.88 |  | 13.39  | 30.11  | 45.21  | 56.68 | 65.20 | 71.73  | 76.24  |
|  | C | 16.74 AB | 33.98 AB | 51.34 | 62.61 |  | 13.59  | 30.67 AB  | 46.02 AB | 56.17 AB | 62.84 AB | 67.98 AB | 71.67 AB  |
| FL 1000 | D1µl | 16.19  | 33.84  | 51.24 | 62.29 |  | 13.41 | 30.29 | 46.93  | **59.52** a | **68.48** a | **74.77** a | **78.82** a |
|  | C | 16.22 AB | 33.72 AB | 51.17 | 62.23 |  | 13.36 | 30.14 AB | 45.80 AB  | **55.95** AB b | **62.20** AB b | **66.97** AB b | **70.19** AB b |
| FL 1500 | D1µl | 16.13  | 33.34  | 51.35 | 62.50 |  | 13.20 | 30.39 | 47.02 | **60.18** a | **69.33** a | **75.56** a | **79.72** a |
|  | C | 16.15 B | 33.09 B | 51.00 | 62.14 |  | 13.26 | 30.48 AB | 46.15 AB | **56.81** A b | **63.64** AB b | **68.41** AB b | **71.62** AB b |
| NL | D1µl | 17.17  | 35.08  | 52.17 | 62.83 |  | 13.34 | 29.46 | 43.29 | 52.74  | 59.66  | 66.03 | 70.55 |
|  | C | 17.11 AB | 35.00 A | 52.16 | 63.12 |  | 13.24 | 29.49 B | 43.47 B | 52.43 B  | 58.67 B | 64.01 B | 67.79 B |
| s.e. |  | 0.39 | 0.89 | 1.40 | 1.53 |  | 0.46 | 0.32 | 0.85 | 1.60 | 2.14 | 2.29 | 2.26 |

**Table S4. Estimated covariance parameter from Model 1 in Series W, G and L.** The values shown represent the estimated variance parametersfor the random factors in Model 1. Trait is the root length at day 7 (Series W and G) or at day 8 (Series L).

|  |  |  |  |
| --- | --- | --- | --- |
| Factor | Series W | Series G | Series L |
| Trial | 11.2 | 1.9 | 5.9 |
| Trial × Dose | 0 | 1.3 | 11.2 |
| Trial × Test factor1 | 19.0 | 15.2 | 8.6 |
| Trial × Test factor × Block | 2.3 | 6.4 | 5.7 |
| Residual | 4ml: 17.8 25 ml: 55.86 ml: 88.5 | 140.4 | 91.9 |

1 Series W: *water volume*; Series G: *laying time*; Series L: *light*

2 Variance heterogeneity for the factor *volume* was considered in Model 1.

**Table S5. Statistical evaluation of individual hanger in series W, G and L.** The values shown represent the p-values for the factor *dose* of the individual analyses (Wald F-test of Model 4 described in Section 1.3). Significant results are shown in bold (p < 0.05). Trait is the root length at day 7 (Series W and G) or at day 8 (Series L).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Series | Factor |  |  |  | Trial |  |  |  |  |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| W | 4 ml 1 | 0.94 | 0.51 | 0.29 | 0.20 |  |  |  |  |
|  | 5 ml | 0.55 | **0.03** (+)a | **0.003** (+)a b | 0.95 |  |  |  |  |
|  | 6 ml | **0.001** (+)a | 0.21 | **0.03** | 0.91 |  |  |  |  |
| G | 1 min | **0.004** (-)a | 0.76 | 0.82 | 0.15 | 0.76 | 0.26 | 0.07 | 0.69 |
|  | 20 min | **0.005** (+)a | **0.007** (+)b | 0.28 | 0.64 | 0.45 | 0.83 | 0.51 | 0.69 |
|  | 40 min | 0.08 | **<0.001** (+)a b | 0.27 | 0.76 | **0.03** (+)a | **0.02** (+)a | 0.20 | 0.52 |
|  | 60 min | 0.38 | 0.08 | 0.46 | 0.61 | **0.01** (+)a b | 0.66 | 0.66 | **0.007** (+)a b |
| L | NL | 0.37 | 0.62 | **0.01** (+)a | 0.53 | **0.03 (+)a** | 0.19  |  |  |
|  | FL 100 | 0.11 | **0.04** (+)a | **0.005** (+)a | 0.06 | **0.03 (+)**a | 0.22 |  |  |
|  | FL 500 | 0.11 | 0.81 | 0.10 | **< 0.001** (+)a | 0.38 | 0.66 |  |  |
|  | FL 1000 | **< 0.001** (+)a  | 0.06 | **0.001** (+)a | **0.003** (+)a | **0.004** (+)a | 0.09 |  |  |
|  | FL 1500 | **< 0.001** (+)a  | 0.14  | 0.90 | **0.001** (+)a | **< 0.001** (+)a | **0.005** (+)a |  |  |

1 Trait is the root length at day 6.

(+) [(-)]: significant increasing [reducing] effect compared to Control (Tukey-Kramer test, p < 0.05, *n* = 20).

a [b]: D1µl [D0.1µ] differs significantly to Control

**Table S6.** Number of discarded bags and seeds.

|  |  |  |  |
| --- | --- | --- | --- |
| Series | Treatment | Discarded bags | Discarded seeds |
| W | D0.1µl | 0 (0%) | 294 (7.7%) |
|  | D1µl | 1 (0.4%) | 330 (8.6%) |
|  | C | 0 (0%) | 305 (7.9%) |
| G | D0.1µl | 1 (0.2%) | 695 (6.8%) |
|  | D1µl | 1 (0.2%) | 748 (7.3%) |
|  | C | 1 (0.2%) | 722 (7.1%) |
| L | D1µl | 0 (0%) | 1,109 (11.6%) |
|  | C | 0 (0%) | 1,107 (11.5%) |

**Table S7.** *P-*values of the fixed effects with number of excluded seedlings as trait.

|  |  |  |  |
| --- | --- | --- | --- |
| Series | W | G | L |
| *Dose*  | 0.28 | 0.28 | 0.93 |
| *Test factor1* | 0.34 | 0.003 | 0.76 |
| *Dose* x *Test factor* | 0.88 | 0.80 | 0.50 |

1 Series W: *volume*, Series G: *laying time*, Series L: *light*

**3. Additional Figures**



**Figure S1.** Root length of cress seedlings in Series W in dependence with the seedling position in bag and the *dose* factor by *volume* levels 4 ml (A), 5 ml (B), and 6 ml (C). Root length at day 6 for (A) and at day 7 for (B) and (C). One point represents the average root length (in mm) based on 73 ± 2 seedlings. Error bars represent ± standard error (*n* = 4). No significant difference between the *dose* treatments by the same seedling position was detected (Modell 2; p < 0.05, Tukey-Kramer-test).



**Figure S2.** Root length of cress seedlings in Series G in dependence with the seedling position in bag and the *dose* factor by *laying time* levels 1 min (A), 20 min (B), 40 min (C), and 60 min (D). One point represents the average root length (in mm) based on 148 ± 4 seedlings. Error bars represent ± standard error (*n* = 8). No significant difference between the *dose* treatments by the same seedling position was detected (Modell 2; p < 0.05, Tukey-Kramer-test).



**Figure S3.** Root length of cress seedlings in Series L in dependence with the seedling position in bag and the *dose* factor by *light* levels FL 100 Lux (A), FL 500 Lux (B), FL 1000 Lux (C), FL 1500 Lux (D), and Natural light (E). One point represents the average root length (in mm) based on 106 ± 3 seedlings. Error bars represent ± standard error (*n* = 6). Asterisks indicate significant differences between D1µl and Control by the same seedling position (Modell 2; p < 0.05, Tukey-Kramer-test).