

# Meta-analysis investigating P and N\*P addition impacts on plant functional traits

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Script that explores the effect of P addition on leaf and whole-plant functional traits using P fertilization and N\*P fertilization experiments. The meta-analysis includes data from the MESI database as of January 07, 2025 and additional experiments compiled by Evan Perkowski.

Script summarizes the number of observations per trait and then conducts a meta-analysis to summarize plant responses to P addition, then conducts a second meta-analysis that summarizes responses when P is added in concert with N.

```
# Libraries
library(dplyr)
library(tidyr)
library(stringr)
library(ggplot2)
library(readr)
library(metafor)
library(MAd)

# MESI data
df_mesi <- read_csv("../data/mesi_main.csv")

# Manual data compilation
df_manual <- read_csv("../data/CNP_meta_data.csv") %>%
  mutate(sampling_year = as.character(sampling_year),
         treatment = ifelse(treatment == FALSE, "f", treatment))

# Merge MESI database with manual data compilation
df_total <- df_mesi %>%
  full_join(df_manual)
```

## Explore data availability in combined dataset for P-fertilization experiments

```
explore_pfert_exps <- df_total %>%

  # fertilisation experiments only
  filter(treatment == "f") %>%

  # P-fertilisation only (without N or K addition)
  filter(npk == "_010")
```

```
head(explore_pfert_exps)
```

```
## # A tibble: 6 x 60
##   db      id    duplicate_id citation    response site  study exp      lat  lon
##   <chr>   <chr> <chr>          <chr>      <chr>   <chr> <chr> <chr> <dbl> <dbl>
## 1 sichuan s885  s885          lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 2 sichuan s888  s888          lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 3 sichuan s879  s879          lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 4 sichuan s882  s882          lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 5 sichuan s385  s385          augustine_~ anpp      0.28~ 0.28~ 0.28~ 0.28 37.9
## 6 sichuan s172  s172          ohalloran_~ agb       -15.~ -15.~ -15.~ -15.4 23.2
## # i 50 more variables: elevation <dbl>, mat <dbl>, map <dbl>,
## #   ecosystem_type <chr>, vegetation_type <chr>, experiment_type <chr>,
## #   community_type <chr>, dominant_species <chr>, growth_form <chr>, age <dbl>,
## #   disturbance_type <chr>, treatment <chr>, npk <chr>, w_t1 <chr>, c_c <dbl>,
## #   c_t <dbl>, d_t <dbl>, d_t2 <dbl>, n_c <dbl>, n_t <dbl>, p_c <dbl>,
## #   p_t <dbl>, k_c <dbl>, k_t <dbl>, i_c <dbl>, i_t <dbl>, i_t2 <dbl>,
## #   s_c <dbl>, s_t <dbl>, w_t2 <dbl>, w_t3 <dbl>, start_year <dbl>, ...
```

```
## How many experiments?
```

```
length(unique(explore_pfert_exps$exp))
```

```
## [1] 219
```

```
## What traits are available?
```

```
unique(explore_pfert_exps$response)
```

```
## [1] "total_biomass_group" "anpp" "agb"
## [4] "total_biomass" "mbc" "soil_total_c"
## [7] "bgb" "leaf_c" "agb_group"
## [10] "anpp_group" "agb_n" "agb_p"
## [13] "soil_total_n" "agb_p_stock" "gpp"
## [16] "fine_root_biomass" "leaf_n_mass" "leaf_p_mass"
## [19] "lai" "mbn" "r_soil"
## [22] "gs" "lwp" "fine_root_production"
## [25] "soil_no3-n_supply_rate" "soil_nh4-n_supply_rate" "soil_p_supply_rate"
## [28] "leaf_biomass_eco" "root_shoot_ratio" "bpe"
## [31] "total_biomass_p_stock" "total_biomass_n_stock" "total_biomass_np"
## [34] "agb_pod_n" "wood_n" "agb_pod_p"
## [37] "wood_p" "agb_grain_n" "agb_grain_p"
## [40] "anpp_grain" "soil_p" "agb_ndvi"
## [43] "agb_height" "bgb_n" "bgb_p"
## [46] "root_n_uptake" "nee" "soil_np"
## [49] "leaf_np" "litter_decomposition" "r_root"
## [52] "nep" "lma" "spad"
## [55] "leaf_n_area" "leaf_p_area" "rgr"
## [58] "amax" "vcmax" "jmax"
## [61] "leaf_pue" "leaf_nue" "leaf_inorg_p"
## [64] "leaf_ester_p" "leaf_nucleic_p" "leaf_lipid_p"
## [67] "leaf_residual_p" "anet" "anet_mass"
## [70] "leaf_metabolic_p" "leaf_structural_p" "leaf_thickness"
## [73] "sla" "ldmc" "rd"
## [76] "stom_lim" "tpu" "asat"
## [79] "cica" "ci" "E"
## [82] "fine_root_p_mass" "coarse_root_p_mass" "stem_p_mass"
```

```
## [85] "total_p_uptake_eff"
```

## Select variables

```
use_response_p <- c("total_biomass",
                    "agb",
                    "bgb",
                    "leaf_n_mass",
                    "leaf_n_area",
                    "leaf_p_mass",
                    "leaf_p_area",
                    "leaf_np",
                    "gs",
                    "lma",
                    "sla",
                    "spad",
                    "amax",
                    "vcmax",
                    "jmax",
                    "leaf_nue",
                    "leaf_pue",
                    "rd",
                    "tpu",
                    "asat",
                    "cica",
                    "ci"
)

pfert_responses <- explore_pfert_exps %>%
  filter(response %in% use_response_p) %>%
  mutate(myvar = response) %>%
  mutate(myvar = ifelse(myvar %in% c("cica", "ci"),
                        "cica", myvar)) %>%
  mutate(myvar = ifelse(myvar %in% c("asat", "amax"),
                        "anet", myvar))

use_vars_p <- unique(pfert_responses$myvar)
```

## Analysis

Calculate "ROM" - the log transformed ratio of means (Hedges et al., 1999; Lajeunesse, 2011) for each observation pair (ambient and elevated).

```
pfert_responses2 <- pfert_responses %>%

  ## keep only essential variables and drop rows containing missing values for
  ## essential variables
  select(id, duplicate_id, exp, myvar, treatment, sampling_year,
         x_t, x_c, sd_t, sd_c, rep_t, rep_c) %>%

  ## Get logarithm of response ratio and its variance
  metafor::escalc(
    measure = "ROM",
    mli = x_t, sdli = sd_t, nli = rep_t,
```

```

    m2i = x_c, sd2i = sd_c, n2i = rep_c,
    data = .,
    append = TRUE,
    var.names = c("logr", "logr_var")
  ) %>%

  ## to keep the output readable from the console output
  as_tibble() %>%

  ## get standard error
  mutate( logr_se = sqrt(logr_var) / sqrt(rep_t) )

head(pfert_responses2)

## # A tibble: 6 x 15
##   id      duplicate_id exp      myvar treatment sampling_year   x_t   x_c sd_t
##   <chr> <chr>          <chr>    <chr> <chr>         <chr>    <dbl> <dbl> <dbl>
## 1 s172  s172          -15.44_2~ agb    f             2         6.29   9.43  5.76
## 2 s3497 s3497          17.25_-8~ agb    f             3        488.   106.   43.7
## 3 s3500 s3500          17.25_-8~ agb    f             3        418.   200.   83.7
## 4 s3503 s3503          17.25_-8~ agb    f             3        247.    80.1  43.7
## 5 s1524 s1524          -18.66_2~ tota~ f             2        76.4   46.7  18.8
## 6 s168  s168          -18.66_2~ agb    f             1        80.0   51.1  19.6
## # i 6 more variables: sd_c <dbl>, rep_t <dbl>, rep_c <dbl>, logr <dbl>,
## #   logr_var <dbl>, logr_se <dbl>

# Aggregate all measurements (multiple years, sampling dates and plots) by experiment (and response var
pfert_responses3 <- pfert_responses2 %>%

  # suggested addition by Kevin, email 02.10.2023 10:03
  dplyr::distinct(duplicate_id, x_t, x_c, .keep_all = TRUE) |>

  filter(!is.na(logr_var) & !is.na(logr)) %>%

  # re-create ID (common ID per experiment and response variable)
  select(-id) %>%
  mutate( id = paste(exp, myvar, sep = "_XXX_") ) %>%

  MAd::agg(
    id = id,
    es = logr,
    var = logr_var,
    cor = 1.0,
    method = "BHHR",
    data = .
  ) %>%

  ## to keep the output readable from the console output
  as_tibble() %>%

  # separate ID again for ease of data use
  mutate( id = str_split(id, "_XXX_") ) %>%
  mutate( exp = purrr::map_chr(id, 1),
    myvar = purrr::map_chr(id, 2) ) %>%

```

```
## rename again
select(exp, myvar, logr = es, logr_var = var) %>%

## add number of observations (sum of plots and repeated samplings)
left_join(
  pfert_responses2 %>%
    group_by(exp, myvar, treatment) %>%
    summarise(n_c = sum(rep_c), n_t = sum(rep_t)),
  by = c("exp", "myvar")
) %>%

## get standard error. Verify if number available observations are identical
## for ambient and elevated. Use N from control here (n_c).
mutate( logr_se = sqrt(logr_var) / sqrt(n_c) ,

        # merge SLA and LMA measurements by taking inverse of logr (keep SE)
        logr = ifelse(myvar == "sla", -logr, logr),
        myvar = ifelse(myvar == "sla", "lma", myvar))
```

## `summarise()` has grouped output by 'exp', 'myvar'. You can override using the  
## `.groups` argument.

```
head(pfert_responses3)
```

```
## # A tibble: 6 x 8
##   exp          myvar      logr logr_var treatment   n_c   n_t logr_se
##   <chr>        <chr>    <dbl>    <dbl> <chr>     <dbl> <dbl> <dbl>
## 1 -15.44_23.25_f agb      -0.405  0.287    f         4     4  0.268
## 2 17.25_-88.77_f agb       1.13  0.0572    f         9     9  0.0797
## 3 -18.66_25.5_f  total_biomass 0.491  0.0285    f         3     3  0.0974
## 4 -18.66_25.5_f agb       0.158  0.418    f         8     8  0.229
## 5 19.6_-155.33_fp bgb       0.142  0.0124    f         3     3  0.0644
## 6 -2.98_-47.52_f agb       0.269  0.000881 f         4     4  0.0148
```

## Meta-analysis

Aggregate log-ratios across multiple experiments, taking into account their respective variance and using the experiment identity as a grouping factor for random intercepts.

```
source("../helper_fxns/analyse_meta.R")

out_p <- purrr::map(as.list(use_vars_p),
  ~analyse_meta(pfert_responses3 %>%
    rename(var = myvar), nam_target = .))
names(out_p) <- use_vars_p
df_box_p <- purrr::map_dfr(out_p, "df_box") |>
  left_join(
    pfert_responses3 |>
      group_by(myvar) |>
      summarise(logr_min = min(logr), logr_max = max(logr)) |>
      rename(var = myvar),
    by = "var"
  )
saveRDS(df_box_p, file = paste0(here::here(), "df_box_pfert.rds"))
```

## Final data size

Number of data points (plot-level measurements) per variable:

```
pfert_responses3 %>%
  group_by(myvar) %>%
  summarise(n_plots = sum(n_c, na.rm = TRUE), n_exp = n()) %>%
  rename("Variable"="myvar", "N observations"="n_plots", "N experiments"="n_exp") %>%
  knitr::kable()
```

Variable	N observations	N experiments
agb	827	97
anet	282	3
bgb	159	27
cica	119	2
gs	289	5
jmax	515	8
leaf_n_area	746	31
leaf_n_mass	796	38
leaf_np	710	35
leaf_nue	427	5
leaf_p_area	746	31
leaf_p_mass	806	39
leaf_pue	427	5
lma	788	34
rd	343	4
spad	169	4
total_biomass	101	14
tpu	130	3
vcmax	515	8

Number of data points (plot-level measurements) per experiment:

```
pfert_responses3 %>%
  group_by(exp) %>%
  summarise(n_plots = sum(n_c), n_exp = n()) %>%
  rename_("Experiment"="exp", "N observations"="n_plots", "N experiments"="n_exp") %>%
  knitr::kable()
```

Experiment	N observations	N experiments
-15.44_23.25_f	4	1
-18.66_25.5_f	11	2
-2.98_-47.52_f	4	1
-21.65_21.81_f	3	1
-22.283_117.666_f	6	1
-22.41_21.71_f	4	1
-22.78_31.25_f	16	2
-23.75_31.43_f	16	2
-24.17_21.89_f	7	2
-24.4_31.75_f	16	2
-25.12_31.23_f	16	2
-25.29_31.91_f	16	2
-3.5_36_f	6	1
-3.95_-79.03_f	12	2

Experiment	N observations	N experiments
-33.35_150.3_f	9	3
-35.73_-58.05_f	12	1
17.25_-88.77_f	9	1
19.6_-155.33_fp	3	1
22.13_-159.63_f	11	1
26.52_109.78_fp	3	1
31.37_90.02_f	24	1
31.3_-81.28_f	4	1
31.55_-81.78_f	4	1
32.54_-116.7_fp	12	1
33.7_120.3_f	15	1
34.92_102.88_f2p	5	1
34.92_102.88_f3p	5	1
34.92_102.88_fp	5	1
37.25_-121.75_forb_fp	3	1
37.25_-121.75_grass_fp	3	1
37.48_101.2_fp	18	3
37.55_-122.3_f	3	1
37.6_101.32_fp	20	3
37.87_-122.52_f	18	2
39.25_-121.28_fp	20	1
39.75_-74.75_fp	4	1
41.35_36.25_fp	4	1
41.35_36.25_fp2	4	1
41.62_-71.32_fp	8	2
42.28_-85.58_f	6	2
42.58_122.21_fp	12	2
44.8_116.03_fp	6	2
47.57_7.6_f	6	1
51.85_5.62_fp	3	1
52.07_5.58_fp	3	1
52.37_5.1_fp	6	1
52.5_5.7_fp	6	1
53.83_-8.83_fp	10	1
54.63_8.83_fp	5	1
64.83_-147.72_fp	3	1
69.43_-133.02_fp	10	1
69.43_-133.02_fp2	10	1
9.6_-79.5_f	3	1
Bloomfield_2014_fp	1505	14
Firn_2019_bldr.us_fnp	24	6
Firn_2019_bnch.us_fnp	54	6
Firn_2019_bogong.au_fnp	72	6
Firn_2019_burrawan.au_fnp	78	6
Firn_2019_cbgb.us_fnp	54	6
Firn_2019_comp.pt_fnp	78	6
Firn_2019_cowi.ca_fnp	54	6
Firn_2019_elliott.us_fnp	54	6
Firn_2019_frue.ch_fnp	54	6
Firn_2019_gilb.za_fnp	54	6
Firn_2019_hopl.us_fnp	54	6
Firn_2019_kiny.au_fnp	27	3

Experiment	N observations	N experiments
Firn_2019_konz.us_fnp	54	6
Firn_2019_lancaster.uk_fnp	54	6
Firn_2019_look.us_fnp	54	6
Firn_2019_mcla.us_fnp	54	6
Firn_2019_mtca.au_fnp	56	4
Firn_2019_sage.us_fnp	54	6
Firn_2019_saline.us_fnp	10	1
Firn_2019_sgs.us_fnp	54	6
Firn_2019_shps.us_fnp	42	6
Firn_2019_sier.us_fnp	54	6
Firn_2019_smith.us_fnp	54	6
Firn_2019_summ.za_fnp	72	6
Firn_2019_unc.us_fnp	54	6
Firn_2019_valm.ch_fnp	126	6
Zavisic_2018_fp	38	3
alpflix_fp	60	1
bennekom_drained_fp	5	1
bennekom_undrained_fp	5	1
bordeaux_fp	40	3
bown_2007_fnp	80	8
buitengoor_1992_fp	5	1
buitengoor_1993_fp	5	1
carswell_2005_fnp	80	4
crous_2017_fnp	2640	12
daqinggou_fp	12	2
drentsche_aa_drained_fp	5	1
drentsche_aa_wet_fp	5	1
ewenke_f_p	6	1
fan_2024_fp	627	11
flottbek_fp	30	1
gusewell_s1_fp	4	1
gusewell_s2_fp	4	1
gusewell_t1_fp	4	1
gusewell_t2_fp	4	1
gusewell_v1_fp	4	1
gusewell_v2_fp	4	1
gusewell_v3_fp	4	1
gusewell_w1_p	4	1
gusewell_w2_p	4	1
gusewell_w3_p	4	1
gusewell_w4_p	4	1
hol_kortenhoef_fp	5	1
ingers_ng_2006np_fp	6	1
indoneisa_f	54	3
kansasf_fp	12	1
kansask_fp	12	1
katelijne_2016_fp	5	1
katelijne_2017_f2	10	1
katelijne_2017_f3	10	1
kisa_grahamiana_f	20	1
luneburg_field_2006_fp	10	1
luneburg_field_2008_fp	10	1



Experiment	N observations	N experiments
lunenburg_gh_drought_molinia_fp	NA	2
lunenburg_gh_fert_molinia_fp	10	1
michigan_underc_bog_fp	19	1
michigan_underc_intermfen_fp	17	1
michigan_underc_richfen_fp	20	1
mo_2019_fnp	175	7
mo_2021_fnp	10	2
molenpolder_fp	5	1
nashfield_pooled_fp	8	2
niwot_ridge2_dm_fp	5	1
niwot_ridge2_wm_fp	5	1
sanjiang_mire_pfert_fp	9	1
sanjiang_mire_pfert_fp2	9	1
sanjiang_mire_pfert_fp3	9	1
sanpedro_fp	6	3
schiermonnikoog_old_fp	12	1
schiermonnikoog_old_fp2	12	1
schiermonnikoog_young_fp	12	1
schiermonnikoog_young_fp2	12	1
shaaxi_330_fp	3	1
shaaxi_6_fp	3	1
tambopata_fp	6	3
teberda_fp	8	1
tono_fp	6	3
toolik_nonacidic_fp	6	2
verryckt_2022_nou_fnp	470	10
verryckt_2022_par_fnp	410	10
warren_2002_fp	30	2
warren_2011_fp	120	8
wayqecha_fp	6	3
westbroek_polder_fp	5	1
yu_2022_fnp	120	5
yucatan_marsh_highsalinity_fp	30	1
yucatan_marsh_lowsalinity_fp	30	1
yucatan_marsh_mediumsalityity_fp	30	1
zwarte_beek_drained_fp	5	1
zwarte_beek_wet_fp	5	1

Some quick plots:

```
pfert_responses3 <- pfert_responses3 %>%
  mutate(myvar = factor(myvar, levels = c("bgb", "agb", "total_biomass", "leaf_pue",
                                           "leaf_nue", "rd", "tpu", "jmax", "vcmax",
                                           "cica", "gs", "anet", "spad", "leaf_np",
                                           "leaf_p_area", "leaf_p_mass", "leaf_n_area",
                                           "leaf_n_mass", "lma")))

meta_plot <- ggplot(data = subset(pfert_responses3, myvar != "cica"),
  aes(x = myvar, y = logr)) +
  geom_jitter(color = rgb(0,0,0,0.3),
    aes( size = 1/logr_se ),
```

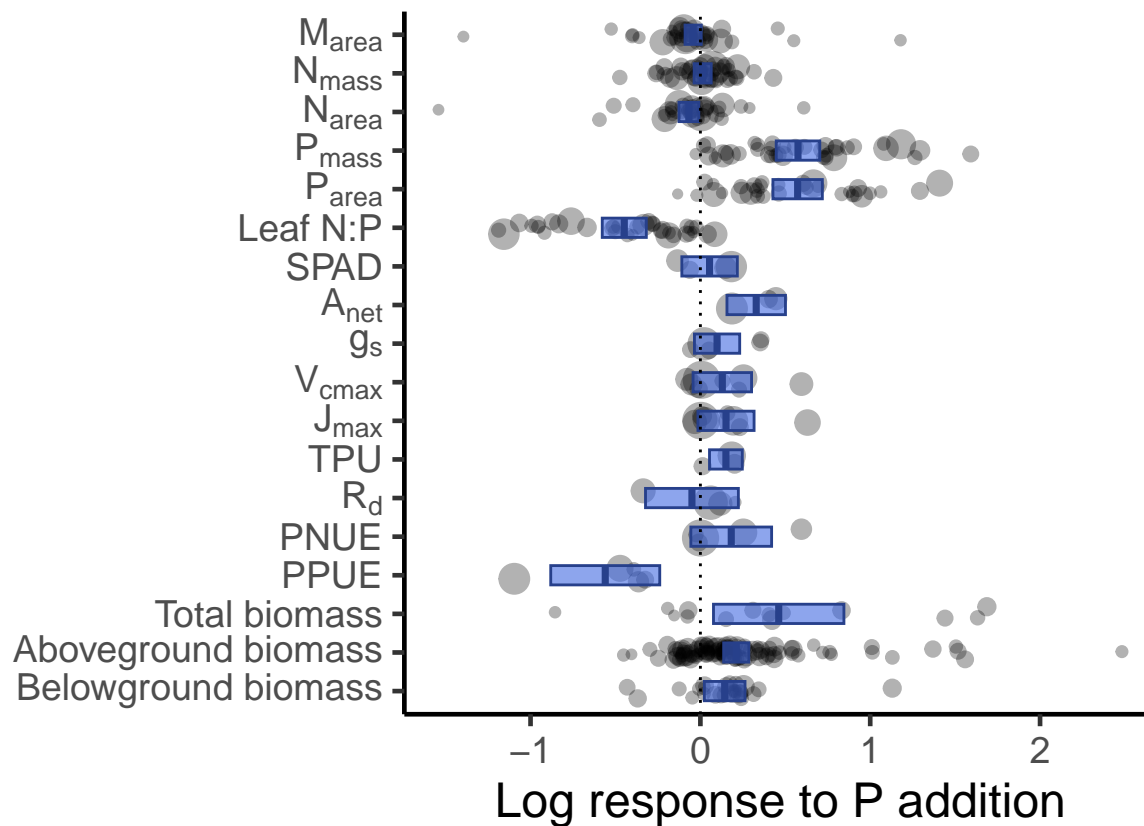
```

    position = position_jitter(w = 0.2, h = 0),
    show.legend = FALSE) +
geom_crossbar( data = df_box_p %>% drop_na(var),
  aes(x = var, y = middle, ymin = ymin, ymax = ymax),
  fill = "royalblue",
  color = "royalblue4",
  alpha = 0.6,
  width = 0.5 ) +
geom_hline( yintercept = 0.0, linewidth = 0.5, linetype = "dotted" ) +
scale_x_discrete(labels = c("Belowground biomass",
  "Aboveground biomass",
  "Total biomass",
  "PPUE",
  "PNUE",
  expression("R"["d"]),
  "TPU",
  expression("J"["max"]),
  expression("V"["cmax"]),
  expression("g"["s"]),
  expression("A"["net"]),
  "SPAD",
  "Leaf N:P",
  expression("P"["area"]),
  expression("P"["mass"]),
  expression("N"["area"]),
  expression("N"["mass"]),
  expression("M"["area"]))) +

#scale_x_discrete("", labels = mylabl) +
labs(x = "",
  y = "Log response to P addition") +
coord_flip() +
theme_classic(base_size = 18)
meta_plot

```

```
## Warning: Removed 2 rows containing missing values (`geom_point()`).
```



## Explore data availability in combined dataset for N\*P-fertilization experiments

```
explore_npfert_exps <- df_total %>%

# fertilisation experiments only
filter(treatment == "f") %>%

# P-fertilization in concert with N-fertilization (without K addition)
filter(npk == "_110")

head(explore_npfert_exps)
```

```
## # A tibble: 6 x 60
##   db      id  duplicate_id citation      response site  study exp      lat  lon
##   <chr>   <chr> <chr>          <chr>        <chr>    <chr> <chr> <chr>  <dbl> <dbl>
## 1 sichuan s886 s886      lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 2 sichuan s889 s889      lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 3 sichuan s880 s880      lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 4 sichuan s883 s883      lawrence_2~ total_b~ 0.12~ 0.12~ 0.12~ 0.12 110.
## 5 sichuan s386 s386      augustine_~ anpp    0.28~ 0.28~ 0.28~ 0.28 37.9
## 6 sichuan s171 s171      ohalloran_~ agb     -15.~ -15.~ -15.~ -15.4 23.2
## # i 50 more variables: elevation <dbl>, mat <dbl>, map <dbl>,
## # ecosystem_type <chr>, vegetation_type <chr>, experiment_type <chr>,
## # community_type <chr>, dominant_species <chr>, growth_form <chr>, age <dbl>,
## # disturbance_type <chr>, treatment <chr>, npk <chr>, w_t1 <chr>, c_c <dbl>,
```

```
## #   c_t <dbl>, d_t <dbl>, d_t2 <dbl>, n_c <dbl>, n_t <dbl>, p_c <dbl>,
## #   p_t <dbl>, k_c <dbl>, k_t <dbl>, i_c <dbl>, i_t <dbl>, i_t2 <dbl>,
## #   s_c <dbl>, s_t <dbl>, w_t2 <dbl>, w_t3 <dbl>, start_year <dbl>, ...
```

```
## How many experiments?
```

```
length(unique(explore_npfert_exps$exp))
```

```
## [1] 251
```

```
## What traits are available?
```

```
unique(explore_npfert_exps$response)
```

```
## [1] "total_biomass_group"      "anpp"
## [3] "agb"                     "total_biomass"
## [5] "agb_c"                   "bgb_c"
## [7] "soil_total_c"            "mbc"
## [9] "bgb"                     "leaf_c"
## [11] "leaf_biomass_eco"        "agb_group"
## [13] "stem_biomass"            "agb_n"
## [15] "agb_p"                   "soil_total_n"
## [17] "agb_p_stock"             "gpp"
## [19] "fine_root_biomass"       "soil_no3"
## [21] "r_soil"                  "total_biomass_production"
## [23] "soil_total_cn"           "root_production"
## [25] "soc"                     "lwp"
## [27] "gs"                      "soil_no3_leaching"
## [29] "soil_nh4"                "soil_drp"
## [31] "soil_p"                  "fine_root_production"
## [33] "soil_no3-n_supply_rate"  "soil_nh4-n_supply_rate"
## [35] "soil_p_supply_rate"      "root_shoot_ratio"
## [37] "bpe"                     "total_biomass_np"
## [39] "agb_cn"                  "agb_cp"
## [41] "agb_ndvi"                "agb_height"
## [43] "bgb_n"                   "bgb_p"
## [45] "root_n_uptake"           "nee"
## [47] "swc"                     "soil_phkcl"
## [49] "soil_pbray"              "som_cn"
## [51] "soil_np"                 "leaf_n_mass"
## [53] "leaf_p_mass"             "leaf_np"
## [55] "litter_decomposition"    "r_root"
## [57] "nep"                     "agb_k_stock"
## [59] "anet"                    "anet_mass"
## [61] "lma"                     "leaf_n_area"
## [63] "leaf_p_area"             "leaf_nue"
## [65] "leaf_pue"                "leaf_metabolic_p"
## [67] "leaf_nucleic_p"          "leaf_structural_p"
## [69] "leaf_residual_p"         "spad"
## [71] "leaf_thickness"          "sla"
## [73] "ldmc"                    "vcmax"
## [75] "jmax"                    "rd"
## [77] "stom_lim"                "tpu"
```

Select variables

```

use_response_np <- c("total_biomass",
                    "agb",
                    "bgb",
                    "leaf_n_mass",
                    "leaf_n_area",
                    "leaf_p_mass",
                    "leaf_p_area",
                    "leaf_np",
                    "gs",
                    "lma",
                    "sla",
                    "spad",
                    "amax",
                    "vcmax",
                    "jmax",
                    "leaf_nue",
                    "leaf_pue",
                    "rd",
                    "tpu",
                    "asat",
                    "cica",
                    "ci"
)

npfert_responses <- explore_npfert_exps %>%
  filter(response %in% use_response_np) %>%
  mutate(myvar = response) %>%
  mutate(myvar = ifelse(myvar %in% c("cica", "ci"),
                        "cica", myvar)) %>%
  mutate(myvar = ifelse(myvar %in% c("asat", "amax"),
                        "anet", myvar))

use_vars_np <- unique(npfert_responses$myvar)

```

## Analysis

Calculate "ROM" - the log transformed ratio of means (Hedges et al., 1999; Lajeunesse, 2011) for each observation pair (ambient and elevated).

```

npfert_responses2 <- npfert_responses %>%

  ## keep only essential variables and drop rows containing missing values for
  ## essential variables
  select(id, duplicate_id, exp, myvar, treatment, sampling_year,
         x_t, x_c, sd_t, sd_c, rep_t, rep_c) %>%

  ## Get logarithm of response ratio and its variance
  metafor::escalc(
    measure = "ROM",
    m1i = x_t, sd1i = sd_t, n1i = rep_t,
    m2i = x_c, sd2i = sd_c, n2i = rep_c,
    data = .,
    append = TRUE,
    var.names = c("logr", "logr_var")
  )

```

```

) %>%

## to keep the output readable from the console output
as_tibble() %>%

## get standard error
mutate( logr_se = sqrt(logr_var) / sqrt(rep_t) )

head(npfert_responses2)

## # A tibble: 6 x 15
##   id      duplicate_id exp      myvar treatment sampling_year   x_t   x_c sd_t
##   <chr> <chr>          <chr>    <chr> <chr>      <chr>      <dbl> <dbl> <dbl>
## 1 s171  s171          -15.44_2~ agb    f          2          8.38  9.43  5.76
## 2 s3499 s3499          17.25_-8~ agb    f          3         506.  106.  40.0
## 3 s3502 s3502          17.25_-8~ agb    f          3         364.  200.  76.4
## 4 s3505 s3505          17.25_-8~ agb    f          3         262.   80.1  76.4
## 5 s1525 s1525          -18.66_2~ tota~ f          2          59.5  46.7  27.1
## 6 s169  s169          -18.66_2~ agb    f          1         163.   51.1  64.3
## # i 6 more variables: sd_c <dbl>, rep_t <dbl>, rep_c <dbl>, logr <dbl>,
## #   logr_var <dbl>, logr_se <dbl>

# Aggregate all measurements (multiple years, sampling dates and plots) by experiment (and response var
npfert_responses3 <- npfert_responses2 %>%

  # suggested addition by Kevin, email 02.10.2023 10:03
  dplyr::distinct(duplicate_id, x_t, x_c, .keep_all = TRUE) |>

  filter(!is.na(logr_var) & !is.na(logr)) %>%

  # re-create ID (common ID per experiment and response variable)
  select(-id) %>%
  mutate( id = paste(exp, myvar, sep = "_XXX_")) %>%

  MAD::agg(
    id = id,
    es = logr,
    var = logr_var,
    cor = 1.0,
    method = "BHHR",
    data = .
  ) %>%

  ## to keep the output readable from the console output
  as_tibble() %>%

  # separate ID again for ease of data use
  mutate( id = str_split(id, "_XXX_") ) %>%
  mutate( exp = purrr::map_chr(id, 1),
    myvar = purrr::map_chr(id, 2) ) %>%

  ## rename again
  select(exp, myvar, logr = es, logr_var = var) %>%

```

```
## add number of observations (sum of plots and repeated samplings)
left_join(
  npfert_responses2 %>%
    group_by(exp, myvar, treatment) %>%
    summarise(n_c = sum(rep_c), n_t = sum(rep_t)),
  by = c("exp", "myvar")
) %>%

## get standard error. Verify if number available observations are identical
## for ambient and elevated. Use N from control here (n_c).
mutate( logr_se = sqrt(logr_var) / sqrt(n_c) ,

        # merge SLA and LMA measurements by taking inverse of logr (keep SE)
        logr = ifelse(myvar == "sla", -logr, logr),
        myvar = ifelse(myvar == "sla", "lma", myvar))

## `summarise()` has grouped output by 'exp', 'myvar'. You can override using the
## `.groups` argument.
head(npfert_responses3)
```

```
## # A tibble: 6 x 8
##   exp          myvar      logr logr_var treatment    n_c    n_t logr_se
##   <chr>        <chr>    <dbl>    <dbl>    <chr>    <dbl> <dbl>    <dbl>
## 1 -15.44_23.25_f agb      -0.118  0.195      f         4     4    0.221
## 2 17.25_-88.77_f agb       1.11  0.0634     f         9     9    0.0839
## 3 -18.66_25.5_f total_biomass 0.242  0.0776     f         3     3    0.161
## 4 -18.66_25.5_f agb       1.01  0.287      f         8     8    0.190
## 5 19.6_-155.33_fnp bgb      0.636  0.0174     f         3     3    0.0762
## 6 -2.98_-47.52_f agb      0.245  0.000560    f         4     4    0.0118
```

## Meta-analysis

Aggregate log-ratios across multiple experiments, taking into account their respective variance and using the experiment identity as a grouping factor for random intercepts.

```
out_np <- purrr::map(as.list(use_vars_np),
  ~analyse_meta(npfert_responses3 %>%
    rename(var = myvar), nam_target = .))
names(out_np) <- use_vars_np
df_box_np <- purrr::map_dfr(out_np, "df_box") |>
  left_join(
    npfert_responses3 |>
      group_by(myvar) |>
      summarise(logr_min = min(logr), logr_max = max(logr)) |>
      rename(var = myvar),
    by = "var"
  )
saveRDS(df_box_np, file = paste0(here::here(), "df_box_npfert.rds"))
```

## Final data size

Number of data points (plot-level measurements) per variable:

```
npfert_responses3 %>%
  group_by(myvar) %>%
  summarise(n_plots = sum(n_c, na.rm = TRUE), n_exp = n()) %>%
  rename("Variable"="myvar", "N observations"="n_plots", "N experiments"="n_exp") %>%
  knitr::kable()
```

Variable	N observations	N experiments
agb	1348	111
bgb	294	39
gs	255	3
jmax	338	5
leaf_n_area	569	28
leaf_n_mass	619	35
leaf_np	590	33
leaf_nue	265	3
leaf_p_area	569	28
leaf_p_mass	619	35
leaf_pue	265	3
lma	626	32
rd	308	3
spad	112	3
total_biomass	90	14
tpu	10	1
vcmax	338	5

Number of data points (plot-level measurements) per experiment:

```
npfert_responses3 %>%
  group_by(exp) %>%
  summarise(n_plots = sum(n_c), n_exp = n()) %>%
  rename_("Experiment"="exp", "N observations"="n_plots", "N experiments"="n_exp") %>%
  knitr::kable()
```

Experiment	N observations	N experiments
-15.44_23.25_f	4	1
-18.66_25.5_f	11	2
-2.98_-47.52_f	4	1
-21.65_21.81_f	3	1
-22.283_117.666_f	6	1
-22.41_21.71_f	4	1
-22.78_31.25_f	16	2
-23.75_31.43_f	16	2
-24.17_21.89_f	7	2
-24.4_31.75_f	16	2
-25.12_31.23_f	16	2
-25.29_31.91_f	16	2
-3.5_36_f	6	1
-3.95_-79.03_f	12	2
17.25_-88.77_f	9	1
19.6_-155.33_fnp	3	1
22.13_-159.63_f	11	1
26.52_109.78_fn2p	3	1



Experiment	N observations	N experiments
31.37_90.02_f	48	1
31.3_-81.28_f	4	1
31.42_-88.45_f	8	2
31.55_-81.78_f	4	1
32.54_-116.7_fnp	12	1
33.7_120.3_f	15	1
34.92_102.88_f2np	5	1
34.92_102.88_f3np	5	1
34.92_102.88_fnp	5	1
35.97_101.88_f	40	2
37.25_-121.75_forb_fnp	3	1
37.25_-121.75_grass_fnp	3	1
37.48_101.2_fnp	18	3
37.55_-122.3_f	3	1
37.6_101.32_fnp	20	3
37.87_-122.52_f	14	2
39.25_-121.28_fnp	20	1
41.35_36.25_fn2p	4	1
41.35_36.25_fn2p2	4	1
41.35_36.25_fn3p	4	1
41.35_36.25_fn3p2	4	1
41.35_36.25_fnp	4	1
41.35_36.25_fnp2	4	1
41.62_-71.32_fnp	8	2
42.58_122.21_fnp	12	2
44.8_116.03_fnp	24	2
51.85_5.62_fnp	3	1
52.07_5.58_fnp	3	1
52.37_5.1_fnp	6	1
52.5_5.7_fnp	6	1
53.83_-8.83_fnp	10	1
54.63_8.83_fnp	5	1
64.83_-147.72_fnp	3	1
68.2_-149.6_f	16	2
68.38_-104.54_f	4	1
69.43_-133.02_fn2p2	10	1
69.43_-133.02_fnp	10	1
9.6_-79.5_f	3	1
Firn_2019_bldr.us_fnp	24	6
Firn_2019_bnch.us_fnp	54	6
Firn_2019_bogong.au_fnp	72	6
Firn_2019_burrawan.au_fnp	78	6
Firn_2019_cbgb.us_fnp	54	6
Firn_2019_comp.pt_fnp	78	6
Firn_2019_cowi.ca_fnp	54	6
Firn_2019_elliot.us_fnp	54	6
Firn_2019_frue.ch_fnp	54	6
Firn_2019_gilb.za_fnp	54	6
Firn_2019_hopl.us_fnp	54	6
Firn_2019_kiny.au_fnp	27	3
Firn_2019_konz.us_fnp	54	6
Firn_2019_lancaster.uk_fnp	54	6

Experiment	N observations	N experiments
Firn_2019_look.us_fnp	54	6
Firn_2019_mcla.us_fnp	54	6
Firn_2019_mtca.au_fnp	56	4
Firn_2019_sage.us_fnp	54	6
Firn_2019_saline.us_fnp	10	1
Firn_2019_sgs.us_fnp	54	6
Firn_2019_shps.us_fnp	42	6
Firn_2019_sier.us_fnp	54	6
Firn_2019_smith.us_fnp	54	6
Firn_2019_summ.za_fnp	72	6
Firn_2019_unc.us_fnp	54	6
Firn_2019_valm.ch_fnp	126	6
alpflix_fnp	60	1
bennekom_drained_fnp	5	1
bennekom_undrained_fnp	5	1
bown_2007_fnp	80	8
buitengoor_1992_fnp	5	1
carswell_2005_fnp	80	4
crous_2017_fnp	2640	12
cuiliugou_f	80	1
cuiliugou_f2	72	1
cuiliugou_f3	72	1
damxung_f	30	1
damxung_f2	30	1
daqinggou_fnp	12	2
drentsche_aa_drained_fnp	5	1
drentsche_aa_wet_fnp	5	1
duolun15_fn2p	4	1
duolun15_fnp	4	1
duolun1_f	4	1
escambia_county_f	8	2
ewenke_f_np	6	1
flottbek_fnfp	25	1
gusewell_s1_fnp	4	1
gusewell_s2_fnp	4	1
gusewell_t1_fnp	4	1
gusewell_t2_fnp	4	1
gusewell_v1_fnp	4	1
gusewell_v2_fnp	4	1
gusewell_v3_fnp	4	1
gusewell_w1_fnp	4	1
gusewell_w2_fnp	4	1
gusewell_w3_fnp	4	1
gusewell_w4_fnp	4	1
haibei_fn1p	33	1
haibei_fn1pp	33	1
haibei_fn2p	33	1
haibei_fn2pp	33	1
haibei_fn3p	33	1
haibei_fn3pp	33	1
imgers_ng_2006np_fn2	6	1
imgers_ng_2006np_fn3	6	1

Experiment	N observations	N experiments
ingers_ng_2006np_fn4	6	1
ingers_ng_2006np_fn5	6	1
ingers_ng_2006np_fn6	6	1
ingers_ng_2006np_fp2	6	1
ingers_ng_2006np_fp3	6	1
ingers_ng_2006np_fp4	6	1
ingers_ng_2006np_fp5	6	1
ingers_ng_2006np_fp6	6	1
jingtai_f	32	1
jingtai_f2	32	1
jingtai_f3	32	1
kansasf_fnp	12	1
kansask_fnp	12	1
katelijne_2016_fnp	5	1
katelijne_2017_f	5	1
lunenburg_field_2006_fnp	10	1
lunenburg_field_2008_fnp	10	1
lunenburg_gh_drought_molinia_fnp	NA	2
lunenburg_gh_fert_molinia_fnp	10	1
michigan_underc_bog_fnp	19	1
michigan_underc_intermfen_fnp	17	1
michigan_underc_richfen_fnp	20	1
mo_2019_fnp	175	7
mo_2021_fnp	10	2
nashfield_pooled_fnp	8	2
niwot_ridge2_dm_fnp	10	1
niwot_ridge2_wm_fnp	10	1
sanpedro_fnp	6	3
schiermonnikoog_old_fn2p	12	1
schiermonnikoog_old_fn2p2	12	1
schiermonnikoog_old_fnp	12	1
schiermonnikoog_old_fnp2	12	1
schiermonnikoog_young_fn2p	12	1
schiermonnikoog_young_fn2p2	12	1
schiermonnikoog_young_fnp	12	1
schiermonnikoog_young_fnp2	12	1
shaaxi_330_fnp	3	1
shaaxi_6_fnp	3	1
tambopata_fnp	6	3
teberda_fnp	8	1
tono_fnp	6	3
toolik_acidic_1981_f	4	1
toolik_acidic_f	4	1
toolik_nonacidic_fnp	18	3
toolik_shrub_f	6	3
verryckt_2022_nou_fnp	470	10
verryckt_2022_par_fnp	410	10
wayqecha_fnp	6	3
yu_2022_fnp	120	5
yucatan_marsh_highsalinity_fnp	30	1
yucatan_marsh_lowsalinity_fnp	30	1
yucatan_marsh_mediusalinity_fnp	30	1

Experiment	N observations	N experiments
zwarte_beek_drained_fnp	5	1
zwarte_beek_wet_fnp	5	1

Some quick plots:

```
npfert_responses3 <- npfert_responses3 %>%
  mutate(myvar = factor(myvar, levels = c("bgb", "agb", "total_biomass", "leaf_pue",
                                           "leaf_nue", "rd", "tpu", "jmax", "vcmax",
                                           "cica", "gs", "anet", "spad", "leaf_np",
                                           "leaf_p_area", "leaf_p_mass", "leaf_n_area",
                                           "leaf_n_mass", "lma")))

meta_plot_np <- ggplot(data = subset(npfert_responses3, myvar != "cica"),
  aes(x = myvar, y = logr)) +
  geom_jitter(color = rgb(0,0,0,0.3),
    aes( size = 1/logr_se ),
    position = position_jitter(w = 0.2, h = 0),
    show.legend = FALSE) +
  geom_crossbar( data = df_box_np %>% drop_na(var),
    aes(x = var, y = middle, ymin = ymin, ymax = ymax),
    fill = "royalblue",
    color = "royalblue4",
    alpha = 0.6,
    width = 0.5 ) +
  geom_hline( yintercept = 0.0, linewidth = 0.5, linetype = "dotted" ) +
  scale_x_discrete(labels = c("Belowground biomass",
                              "Aboveground biomass",
                              "Total biomass",
                              "PPUE",
                              "PNUE",
                              expression("R"["d"]),
                              "TPU",
                              expression("J"["max"]),
                              expression("V"["cmax"]),
                              expression("g"["s"]),
                              "SPAD",
                              "Leaf N:P",
                              expression("P"["area"]),
                              expression("P"["mass"]),
                              expression("N"["area"]),
                              expression("N"["mass"]),
                              expression("M"["area"]))) +

  #scale_x_discrete("", labels = mylabl) +
  labs(x = "",
    y = "Log response to N:P addition") +
  coord_flip() +
  theme_classic(base_size = 18)
meta_plot_np
```

```
## Warning: Removed 2 rows containing missing values (`geom_point()`).
```

