

Gut microbiota plasticity is correlated with sustained weight loss on a low-carb or low-fat dietary intervention

Jessica A Grembi^{a,*}, Lan H Nguyen^{b,*}, Thomas D Haggerty^c, Christopher D Gardner^d, Susan P Holmes^e, and Julie Parsonnet^{c,f}

^a*Department of Civil and Environmental Engineering, Stanford University*

^b*Institute for Computational and Mathematical Engineering, Stanford University*

^c*Department of Medicine, Stanford University School of Medicine*

^d*Stanford Prevention Research Center, Department of Medicine, Stanford University School of Medicine*

^e*Department of Statistics, Stanford University*

^f*Department of Health Research and Policy, Stanford University School of Medicine*

**These authors contributed equally*

Abstract

Background Obesity is a complex global health challenge. Although both low-carbohydrate (low-carb) and low-fat diets can lead to weight loss, there is typically substantial variability in weight and related outcomes for both diet approaches among obese but otherwise healthy adults. Elucidating individual characteristics that might contribute to sustained weight loss is critical to developing effective dietary intervention strategies. We hypothesized that structural differences in the gut microbiota explained some portion of the weight loss variability among people randomized to either a low-carb or low-fat diet, possibly related to its effects on dietary compliance.

Results Our study included two staggered cohorts of obese adults enrolled in the Diet Intervention Examining The Factors Interacting with Treatment Success (DIETFITS) study - a randomized clinical trial of either a low-fat or low-carb diet. In the discovery cohort (n=66), 161 pre-diet fecal samples were sequenced in addition to 157 samples collected after 10-weeks of dietary intervention. In the validation cohort (n = 56), 106 pre-diet fecal samples were sequenced. Pre-diet taxonomic features, such as the Prevotella/Bacteroides ratio, correlated to weight loss in the discovery cohort were not confirmed in the validation cohort. The most robust finding in the discovery cohort indicated that gut microbiota plasticity was linked to 12-month weight loss in a diet-dependent manner; subjects with higher sustained weight loss on a low-fat diet had higher pre-diet daily plasticity, whereas those most successful on the low-carb diet had greater microbiota plasticity over 10 weeks of dietary intervention. Unfortunately, because sample frequency and timing was quite different in the validation cohort, the relationship between plasticity and weight loss could not be studied in this group.

Conclusions These findings suggest the potential importance of gut microbiota plasticity in sustained weight loss. We highlight the importance of evaluating kinetic trends and in assessing reproducibility in studies of the gut microbiota.

Keywords low-carb, low-fat, diet, weight loss, microbiota, plasticity

27 Background

28 The global obesity pandemic has claimed one in three American adults and prevalences continue
29 to rise in many other countries as well [46]. Obesity comorbidities (e.g. cardiovascular diseases,
30 cancer, diabetes, and other chronic conditions) cost the US 8.65 billion/yr from loss of productivity
31 due to absenteeism alone [58, 65, 53, 55, 14]. The personal, social and economic costs provide an
32 urgent need for consistently effective, and possibly more personalized, weight-reduction therapies.

33 Different dietary interventions, such as low-carbohydrate (low-carb) and low-fat diets, can lead
34 to weight loss, but not always; there remains substantial variability in diet success outcomes among
35 obese, but otherwise healthy, adults [37]. Additionally, adherence to dietary intervention strategies
36 has remained a major challenge, despite the clear dose-response relationship with weight loss for
37 individuals on both low-carb and low-fat diets [17, 1]. Thus, practitioners have begun to look for
38 individual characteristics (e.g. physiological attributes, cultural or lifestyle characteristics, food
39 preferences, etc.) that could influence an individual's sustained weight loss, possibly by improving
40 adherence to a specific dietary regime.

41 Gut microbiota are highly individualized and intricately involved with the quantity and quality
42 of nutrients extracted from our diets, with direct implications for obesity [19, 62, 26, 41, 60, 59].
43 Microbial metabolites and proteins are known to communicate with the host to influence appetite
44 control [33, 11, 22, 49]. For example, proteins from gut *E. coli* modulate appetite by interacting with
45 antigens involved in host satiety signaling [6]. Byproducts of microbial fermentation (e.g. butyrate
46 and propionate) also stimulate gut hormones that reduce food intake [42, 50]. Studies assessing
47 inter-individual variability in gut microbiota alterations in response to dietary interventions are
48 limited by short-term (<12 weeks) dietary modifications or measurement of metabolic outcomes
49 (e.g. plasma glucose, triglycerides, insulin, cholesterol) rather than weight loss [28]. The one
50 published study that directly investigated the relationship between baseline microbiota structure
51 and long-term weight loss found that a high fecal *Prevotella/Bacteroides* (P/B) ratio (> 0.01 , $n =$
52 15) at baseline resulted in a mean of 1.31kg more weight loss at 6 months than a low P/B ratio
53 (P/B < 0.01 , $n = 21$) [32]. No studies have investigated the impact of microbiota on adherence to

54 a dietary intervention.

55 The DIETFITS study [57, 24] was a randomized trial of 609 adults designed to elucidate predis-
56 posing individual characteristics—genotype, insulin-glucose dynamics, physiological and psychosocial
57 attributes—that contribute to successful 12-month weight loss on *ad libitum* diets designed to be
58 lower in carbs or fat. Within a population subset of DIETFITS, we explored whether attributes of
59 the gut microbiota predisposed individuals to successful 12-month weight loss and how that might
60 have been mediated by dietary adherence. Identifying pre-diet features of the gut microbiota that
61 can predict adherence and/or success on a specific diet might permit personalization of dietary
62 intervention strategies to maximize weight loss.

63 Results

64 Subject demographics and sequencing statistics

65 We recruited subjects from two cohorts of obese adults enrolled in the DIETFITS randomized
66 trial of low-carb and low-fat diets. The cohorts were enrolled approximately six months apart,
67 allowing one to be used for discovery and the second for validation. From each of these cohorts,
68 individuals who a) provided fecal samples prior to initiating the intervention that passed quality
69 filtering (>10,000 high-quality 16S rDNA sequences) and b) completed the one-year intervention
70 were included in our study.

71 The discovery cohort included 66 subjects, of whom 32 (22 female) were randomized to the
72 low-carb diet and 34 (17 female) to the low-fat diet. These 66 subjects provided fecal samples
73 on three consecutive days prior to starting the diet plus three additional daily samples 10 weeks
74 after diet initiation. A sequencing depth of $73,659 \pm 33,380$ reads per sample was obtained from
75 318 fecal samples (161 pre-diet; 157 at 10 weeks). Subject characteristics and dietary information
76 can be found in Table ???. Subjects on the low-carb diet restricted carbs to $22.6 \pm 10.3\%$ of their
77 daily kilocalories (kcal) and lost $8.4 \pm 7.7\%$ of their starting weight after 12 months of dietary
78 intervention whereas those on the low-fat diet restricted fats to $25.3 \pm 5.7\%$ of daily kcal and lost
79 $6.3 \pm 7.7\%$ of their starting weight. Previous definitions of long-term weight loss success [64, 36]

80 were used to categorize subjects based on the percentage of baseline weight lost at 12 months: 20
81 were unsuccessful (US: < 3% weight loss), 25 were moderately successful (MS: 3 – 10% weight loss),
82 and 21 were very successful (VS: > 10% weight loss).

83 The validation cohort was comprised of 56 subjects: 31 (25 female) on the low-carb and 25 (19
84 female) on the low-fat diet. Subject characteristics were comparable between the two cohorts (Table
85 ??), except for the percent weight lost at 12 months on the low-carb diet, which was significantly
86 lower in the validation cohort ($4.8 \pm 6.2\%$ compared to $8.4 \pm 7.7\%$ in the discovery cohort, Welch's t-
87 test $p = 0.045$) and percent of carbs consumed ($31.5 \pm 9.1\%$ compared to $22.6 \pm 10.3\%$ in discovery
88 cohort, Welch's t-test $p = 0.0005$). Subjects were classified to weight loss success groups, as
89 described above, with the following distribution: 24 US, 18 MS, and 14 VS. From these "validation"
90 subjects, fecal samples were collected only prior to the start of the dietary intervention at a median
91 of 12 (IQR 7, 25) days apart. Samples meeting quality criteria ($n = 106$) had a mean sequencing
92 depth of $70,041 \pm 15,664$ reads per sample.

93 **Pre-diet gut microbial community composition does not predict 12-month** 94 **weight loss success**

95 Pre-diet gut microbial community composition varied among subjects, and samples collected from
96 the same individual tended to cluster together in principal coordinates analysis (PCoA) shown
97 in Fig. 1. In the discovery cohort, pre-diet microbiota composition did not cluster by 12-month
98 weight-loss success category (PERMANOVA on Bray-Curtis dissimilarity low-carb: $p = 0.51$; low-
99 fat: $p = 0.81$). The microbiota composition also did not cluster by age, gender, pre-diet weight,
100 body mass index or dietary compliance in the PCoA maps. Similar results were found in the
101 validation cohort (Fig. 1b).

102 **Higher gut microbiota plasticity predicted successful 12-month weight loss**

103 In the discovery cohort, 85% of subjects ($n = 26$ for low-carb, $n = 30$ for low-fat) provided two or
104 three fecal samples on consecutive days prior to the start of the intervention. These samples allowed

105 us to quantify subjects' *pre-diet daily microbiota plasticity*, i.e., the amount of daily variability in
106 an individual's microbiota composition, measured with β -diversity metrics. Pre-diet microbiota
107 plasticity was significantly higher for VS subjects on the low-fat diet compared to US subjects
108 (baseline BL v. BL plots in Fig. 2, Wilcoxon rank-sum test $p = 0.033$ for Bray-Curtis dissimilarity).
109 This result was robust to the choice of distance metric (Fig S3) and was repeated on clusters
110 of phylogenetically related ASVs (sharing roughly species- or genus-level sequence similarity; see
111 Methods for details) with similar results (Fig S3b). There was no difference in pre-diet plasticity
112 between weight-loss success groups for the low-carb diet. Consecutive daily samples were also
113 collected after subjects had been on the dietary intervention for approximately 10 weeks; there was
114 no difference in daily plasticity between weight-loss groups at 10 weeks for either diet (10wk v.
115 10wk plots in Fig. 2, Wilcoxon rank-sum test on Bray-Curtis dissimilarity $p = 0.61$ for low-carb,
116 $p = 0.54$ for low-fat).

117 We also quantified *plasticity over ten weeks* in response to the dietary intervention, i.e., the
118 variability between gut microbiota composition before the start of the dietary intervention and
119 after 10 weeks of dieting. The plasticity over ten weeks was computed for subjects ($n = 28$ low-
120 carb, $n = 32$ low-fat) who provided at least one pre-diet sample and another ten weeks after the
121 start of the dietary intervention (77 ± 9 days apart for low-carb diet, 81 ± 14 days for low-fat).
122 For each subject, the average pairwise β -diversity (between each pre-diet and 10-week sample) was
123 calculated. On both diets VS subjects had higher plasticity between their baseline and 10-week
124 fecal microbiota communities than US subjects (BL v. 10wk plots in Fig. 2; Wilcoxon rank-sum
125 test on Bray-Curtis dissimilarity: low-carb $p = 0.017$; low-fat $p = 0.11$). Again, for both diets
126 the observed trends were robust across multiple β -diversity metrics and clustered-ASV data (Fig.
127 S4). Daily pre-diet plasticity was positively correlated with plasticity over ten weeks for subjects
128 on the low-fat diet (Spearman's rank correlation 0.37 for Bray-Curtis, $p = 0.053$). The magnitude
129 of variation between the pre-diet and the 10-week period was higher than day-to-day plasticity at
130 either the baseline or 10-week time point (Fig. 2, Wilcoxon rank-sum test $p < 0.0001$ for both
131 low-carb and low-fat). Although intra-individual plasticity over ten weeks was significantly higher
132 for VS compared to US subjects, inter-individual differences across subjects were still larger in

133 magnitude on both diets (Fig. S5).

134 Within each cohort, pre-diet phylogenetic α -diversity was not different between US and VS
135 subjects (Fig. S1) and was not correlated with dietary compliance on either diet (data not shown).
136 However, for subjects in the discovery cohort, we found a negative correlation between pre-diet
137 plasticity and average α -diversity (mean of all pre-diet samples) for subjects on the low-fat diet
138 (Spearman's rank correlation coefficient -0.42 for Bray-Curtis dissimilarity, $p = 0.021$). This sug-
139 gests that individuals on the low-fat diet with low mean bacterial alpha-diversity tend to exhibit a
140 more variable microbiota composition.

141 The magnitude of gut microbiota plasticity (using any β -diversity metric) was not statistically
142 different between women and men at any time point: pre-diet (Wilcoxon rank-sum test on Bray-
143 Curtis distance $p = 0.86$), at 10-week ($p = 0.35$), or between pre-diet and 10-week ($p = 0.54$).
144 Subjects in the validation cohort did not collect pre-diet fecal samples on consecutive days (median
145 12 [IQR 7,25] days between samples) and fecal samples from 10-weeks into the intervention were
146 not available, so we were unable to validate plasticity as a factor in weight loss success.

147 **Gut microbiota plasticity correlated with dietary compliance in a diet- 148 and sex-dependent manner**

149 Subjects were instructed to strive to restrict nonfiber carbohydrate or fat intake to 20g/d but to
150 titrate over the first few weeks of the study to levels they perceived they could maintain for the rest
151 of their lives. Each diet resulted in a reduced intake of the restricted component (Table ??) but also
152 in the number of total calories consumed. Thus we calculated a proxy measure for adherence to a
153 low-fat or low-carb diet, referred to here as *dietary compliance* and quantified as the reduction in
154 restricted foods (measured as decrease in percentage of total daily kcals consumed from fats/carbs)
155 between baseline (pre-diet) and during the dietary intervention (an average was taken over three
156 unannounced 24-h dietary recalls at each time point conducted during the trial - baseline, 3, 6, and
157 12 months). We conducted sub-group analyses on dietary compliance for men and women, given
158 observed sex-specific differences in correlations between reported dietary compliance and weight
159 loss in the larger DIETFITS study population.

160 Women and men on the low-fat diet exhibited different correlations between dietary compliance
161 and pre-diet daily plasticity (Fig. 3). Men with higher plasticity reduced their fat consumption
162 more (had higher compliance) than men with lower plasticity (Spearman's rank correlation using
163 Bray-Curtis dissimilarity 0.55, $p = 0.02$), whereas women lacked a meaningful correlation between
164 plasticity and dietary compliance (Spearman's rank correlation -0.47, $p = 0.1$). For both women
165 and men on the low-carb diet, no significant correlations were found between dietary compliance
166 and daily pre-diet plasticity. These trends were consistent across several distance metrics (Fig. S6)
167 and using ASV-clusters (data not shown).

168 We suspected that dietary compliance would affect diet-induced microbiota plasticity over ten
169 weeks; more specifically, we expected the gut bacterial community composition of the more adherent
170 subjects to shift more in response to their modified diet on the intervention). We observed highly
171 significant and opposite correlations between men and women on the low-fat diet (see Fig. 3). This
172 was not the case for subjects on the low-carb diet, where again no correlations were noted between
173 diet-induced plasticity and compliance to the prescribed diet for both women and men. Trends
174 were consistent across a variety of distance metrics (Fig. S6).

175 **Pre-diet *Prevotella*/*Bacteroides* ratio correlated with 12-month weight** 176 **loss success on low-carb diet in discovery, but not validation, cohort**

177 In the discovery cohort, subjects on the low-carb diet with VS weight-loss had significantly higher
178 *Prevotella*/*Bacteroides* (P/B) ratio (median = 0.014) compared to US subjects (median = 0.0004;
179 Wilcoxon rank-sum test $p = 0.021$); however, the same was not observed in the validation cohort
180 (VS median P/B ratio = 0.0003; US median = 0.0009; $p = 0.718$; Fig. 4). There was no difference in
181 P/B ratio between US and VS subjects randomized to the low-fat diet for either cohort (discovery:
182 $p = 0.54$; validation: $p = 0.46$).

183 **Differential abundance of sequence clusters not consistently predictive of** 184 **12-month weight loss success**

185 To evaluate patterns of specific members of the pre-diet microbiota that might predict weight
186 loss success, we tested for clusters of phylogenetically related ASVs (sharing roughly species- or
187 genus-level sequence similarity; see Methods section for details on ASV-clustering) that differed in
188 abundance between dieters who achieved VS 12-month weight loss compared to US. We compared
189 within each diet separately, filtering out clusters that were not present in at least 10% of subjects
190 from the focal diet. Using methods allowing for subject as a random effect (`limma` package, see
191 Methods), in the discovery cohort we found one cluster containing 10 Ruminococcaceae ASVs
192 (Cluster94) that was significantly more abundant in US compared to VS subjects on the low-carb
193 diet ($p = 0.0006$) (see Table 2). In contrast, VS subjects had significantly higher abundances of
194 a cluster containing 64 different Ruminococcaceae ASVs (Cluster65, ($p = 0.023$) and a cluster
195 containing 2 *Enterorhabdus* ASVs (Cluster266, $p = 0.025$) compared to US subjects on the low-
196 carb diet. The abundances do not display a proportional linear dose-dependent relationship with
197 percentage weight loss for all subjects, however (Fig. 5), suggesting that these clusters are unlikely
198 to be strong predictors of weight-loss success in a larger population. No clusters were identified
199 as differentially abundant for the low-fat diet. Similar results were obtained when differential
200 abundance testing was performed on the unclustered ASV counts (Fig. S7) and also when a
201 linear model with a continuous predictor corresponding to the percentage weight loss (instead of
202 categorical: VS vs US) was applied. ASVs and ASV-clusters identified as differentially abundant
203 in the discovery cohort were not significant in the validation cohort (Table 2). In most cases, both
204 the magnitude and direction of effect were discordant between cohorts.

205 **Discussion**

206 Our study suggests the potential importance of gut microbiota plasticity in sustained weight loss.
207 The observed patterns in correlations between plasticity and our estimates of dietary compliance
208 could imply sex- and diet-dependent mechanisms. In several earlier studies [15, 20, 68], individual

209 temporal variability of the gut microbiota was seemingly eclipsed by larger-scale variability across
210 body habitats, severe perturbations, or geographically distinct populations, which may have led
211 some to underestimate the importance of plasticity within an individual. However, a study of
212 weekly samples from 85 adults showed that temporal variability is a personalized feature [23]; with
213 some individuals displaying consistently high or low compositional variability. Our results are the
214 first to illustrate that this personalized feature of the microbiota might be relevant to weight loss.

215 Higher pre-diet daily plasticity in gut community composition was observed in subjects attaining
216 higher 12-month weight loss, but only on the low-fat diet. This result was consistent across several
217 β -diversity metrics indicating that microbiota was more plastic both in terms of membership and
218 structure. Of note, no pre-diet microbes were differentially abundant for US compared to VS sub-
219 jects on the low-fat diet, a result that might have been influenced by the increased plasticity found
220 in the latter. In agreement with other studies, we saw a negative correlation with compositional
221 variability and phylogenetic diversity (more diverse communities were less variable) [23, 16, 44].
222 The insurance hypothesis in ecological theory suggests that biologically diverse communities are
223 more resilient as they contain a larger set of community traits/functions that enable them to adapt
224 to changing environments and buffer the system against the loss of species [67]. In our case, we
225 see that higher turnover of microbes might allow for a greater response to the dietary intervention,
226 independent of diversity, possibly by allowing for the alteration of the microbial consortia into one
227 less efficient at extracting nutrients.

228 As this was seen only for low-fat dieters, we hypothesized that increased plasticity might have
229 facilitated responsiveness to the increased carbohydrates and fiber consumed on the low-fat diet
230 [24], making the transition to the new diet easier for subjects in ways that aided adherence through
231 appetite suppression [33, 39, 8, 25, 12, 3, 2]. Our data suggest this is true for men, as we saw positive
232 correlations between plasticity and both weight loss and dietary compliance. Despite magnitudes
233 comparable to men's daily pre-diet plasticity, women on the low-fat diet displayed a negative trend
234 between plasticity and dietary compliance. This suggests that the mechanisms underlying the
235 relationship between plasticity and weight loss might be independent of dietary adherence for this
236 sub-population. Sex hormones play integral roles in appetite regulation [31, 30]. Although we

237 did not analyze hormones directly, it is possible that these signals might be differentially stronger
238 than other anorexigenic signals originating from the gut for women compared to men, possibly
239 explaining some of the sexual dimorphism in our results. Additionally, self-reporting biases might
240 have influenced the 24-hr dietary recalls, and thus our dietary compliance measures, differentially
241 for men and women [29]. We accounted for this by calculating macronutrient intake as a percentage
242 of total energy intake, but these biases were not assessed directly in the survey instrument and thus
243 we were unable to specifically adjust for them.

244 Plasticity of the gut microbiota over the first ten weeks of the dietary intervention was higher
245 for VS compared to US subjects on either diet, but significant only for low-carb dieters. Again, we
246 saw strong diet- and sex-specific differences between plasticity and dietary compliance. Although
247 higher microbiota plasticity was seen in VS low-carb dieters, there was no correlation between
248 plasticity and dietary compliance for either sex, implying that for diets with restricted carbs the
249 microbiota might play a larger role in the proportion of nutrients extracted from foods and less of
250 a role in appetite suppression. The correlations between 10-week plasticity and dietary compliance
251 were positive for men on the low-fat diet and negative for women, again implying that sex-specific
252 differences are likely in our measurement of dietary compliance and/or in the physiological impacts
253 of gut microbiota on appetite suppression.

254 Plasticity of the gut microbiota over ten weeks, and in response to the intervention, was higher
255 for both diets than daily plasticity at either time point. This was expected as dietary changes can
256 have strong influences on taxonomic composition and functional capability of the gut microbial
257 community [21, 19, 38, 56, 45]. However, the non-standardized, self-titrated interventions did not
258 lead to a convergence of subjects to similar gut microbiota communities. After 10 weeks on the
259 dietary intervention, subjects' community membership and structure were still more similar to their
260 own pre-diet communities rather than to other subjects on the same diet. The repeated measures at
261 both time points (pre-diet and 10 weeks) enabled us to observe that the plasticity of the microbial
262 community in response to the diet was higher than daily variability at either time point. As we
263 were unable to evaluate the robustness of these plasticity findings in our validation cohort due
264 to differences in sampling regimes, there remains the need for other studies to corroborate these

265 plasticity findings. Future analyses should be stratified by sex, with specific attention paid to
266 menopausal state of women included.

267 Finally, our study highlighted the importance of validation cohorts in microbiota studies with
268 small to moderate sample sizes. Contrary to our hypothesis, we found no specific taxonomy-related
269 microbial features clearly associated with 12-month weight-loss success. This could be due to
270 our small sample size ($n=25-34$ for any diet-cohort combination), indeed, only $\sim 20\%$ of ASV-
271 clusters were detected in at least half of the subjects on either diet, limiting our statistical power
272 to identify differences between VS and US subjects. Although our sample size was comparable to
273 other published studies investigating the gut microbiota and diet responsiveness [32, 38, 18], our
274 analysis included stringent filtering of samples with at least 10,000 reads and ASV-clusters present
275 in at least 10% of subjects on the focal diet. We also included replicate samples for most subjects
276 in order to reduce false positive identification during differential abundance testing. The majority
277 of pre-diet ASVs and ASV-clusters identified as differentially abundant between weight-loss groups
278 on the low-carb diet were discordant across the discovery and validation cohorts in both magnitude
279 and direction. In addition, our finding from the discovery cohort that subjects on the low-carb diet
280 with higher pre-diet P/B ratio had higher 12-month weight loss was not confirmed in the validation
281 cohort. These inconsistencies could be the result of the significantly lower dietary compliance
282 and weight loss achieved by low-carb dieters in the validation cohort compared to subjects from
283 the discovery cohort. The inclusion of a validation cohort illustrated the challenges in generalizing
284 findings from an initial modest sample size to even a similar population. Future research, specifically
285 designed and powered for the outcome of interest, will be necessary to determine if specific taxa or
286 higher P/B ratio are important for 12-month weight loss in larger populations.

287 **Conclusions**

288 Our study is the first to examine the connection between gut microbiota and 12-month weight
289 loss success on two common diets – low-carb and low-fat – and on the intermediary of dietary
290 compliance. For subjects on the low-fat diet, higher weight loss was observed in subjects with

291 higher pre-diet daily microbiota plasticity but this appears to be mediated by adherence only for
292 men. Low-carb dieters appear to obtain little benefit from daily pre-diet gut microbiota plasticity
293 in terms of weight loss. Higher plasticity of gut microbiota over ten weeks, and in response to
294 the dietary intervention, was seen for subjects who lost the most weight after 12 months of low-
295 carb dieting, but we found no correlations for this group between plasticity and dietary compliance.
296 Despite strong correlations between gut microbiota plasticity over ten weeks and dietary compliance
297 for low-fat dieters, no significant difference in plasticity was noted between VS and US weight loss
298 success groups.

299 Gut microbiota plasticity has not been extensively studied in relation to sustained weight loss
300 or dietary adherence. Here we present data that suggests the plasticity of the gut microbiota
301 may be related to both in a sex-and diet-specific manner. Our work highlights the importance of
302 investigating kinetic trends in gut bacterial community composition, including long-term shifts and
303 daily plasticity, in addition to explicitly seeking static microbiota signatures and patterns, when
304 studying individual differences and predisposition to successful weight loss.

305 **Methods**

306 **Study population and sample collection**

307 Overweight and obese adults in the Diet Intervention Examining The Factors Interacting with
308 Treatment Success (DIETFITS) study enrolled between Fall 2013 and Spring 2014 were approached
309 for inclusion in this study. DIETFITS was a 12-month randomized clinical trial of low-carb and
310 low-fat diets [57, 24] (clinical trial registration NCT01826591). The diets had no specific caloric,
311 fat or carbohydrate restrictions, but instead involved counseling sessions focused on three main
312 components central to sustaining a low-carb or low-fat diet. First, for the initial eight weeks,
313 subjects were instructed to progressively reduce either carbohydrate or fat intake as much as possible
314 (with an objective of achieving 20 g/day), and maintain their lowest possible intake for at least
315 several weeks. Second, after the initial eight weeks they were then encouraged to titrate their intake
316 by increasing fat or carbohydrate consumption by increments of 5-15 g/day and maintaining that

317 intake for a week or more while noting their satisfaction (e.g., satiety, palatability) and weight
318 loss success. After settling on a sustainable target (one that left them feeling full but still able to
319 lose weight), they were asked to maintain that intake level for the remainder of the study. The
320 third component of the study's strategy was promotion of a high-quality diet focused on whole,
321 real foods, that are mostly prepared at home and that contain as many vegetables as possible.
322 Subjects were assigned to attend 22 in-person instructional sessions related to nutrition, behavior,
323 emotions, and physical activity. Participant data, including clinical outcomes, was collected and
324 managed using REDCap electronic data capture tools hosted at Stanford University [27]. Subjects
325 from the discovery cohort were asked to provide self-collected fecal samples from three consecutive
326 days at two separate time points (pre-diet and 10 weeks after initiation of the dietary intervention).
327 Subjects from the validation cohort were asked to provide two self-collected pre-diet fecal samples.
328 Fecal samples were stored at -20°C until delivered to the lab and then at -80°C until processing.
329 A total of 424 fecal samples with sufficient sequencing depth in high-quality reads were collected
330 from 66 discovery cohort and 56 validation cohort subjects who provided complete weight data.

331 **DNA extraction and 16S rRNA gene sequencing**

332 DNA was extracted from 50-150mg fecal material using the Qiagen PowerSoil DNA Isolation Kit
333 (Qiagen, Venlo, The Netherlands) with the following protocol modifications: samples were incu-
334 bated in lysis buffer at 65°C for 10 minutes, bead beating was conducted for 20 minutes, and all
335 subsequent vortexing steps were replaced with gentle but thorough inversions. Extracted DNA was
336 stored at -20°C. The V4 region of the 16S rRNA gene was amplified using the protocol of Caporaso
337 et al. [13]. Briefly, samples were amplified in triplicate 25ul PCRs in 96-well plates with the final
338 volumes per reaction: water 10.9 ul, MasterMix (5 PRIME HotMasterMix) 10 ul, reverse primer
339 0.1 ul, forward primer 1 ul, template DNA 3 ul. Replicates were run simultaneously on 3 thermal
340 cyclers for 30 cycles of: 94°C 45s, 52°C 60s, 72°C 90s with a ten minute extension at 72°C at
341 the end. Amplification was verified by gel electrophoresis on pooled replicates (failures were re-
342 peated) and bands were excised and cleaned (MO BIO UltraClean-htp 96 Well PCR Clean-Up Kit)
343 per manufacturer protocol. DNA was quantified (Invitrogen Quant-iT ds DNA Assay Kit, High

344 Sensitivity) using a microplate reader (FLEXstation II 384-Fluorescent 6), and amplicons were
345 combined in equimolar ratios. This pooled DNA was concentrated via ethanol precipitation and
346 then resuspended in nuclease-free water. DNA libraries were submitted to the Functional Genomics
347 Facility at Stanford University for sequencing on an Illumina MiSeq (2 x 250bp) over 7 separate
348 runs, yielding 73.3M raw reads.

349 **DADA2 Amplicon Sequence Variant (ASV) Sample Inference and Tree** 350 **Building**

351 The DADA2 R package [10] was used for quality filtering, denoising, chimera removal and sequence
352 inference (obtaining sequence counts). Forward reads were trimmed to length 240 in all but one
353 sequencing run (length of 230 due to lower quality); reversed reads, commonly with lower quality,
354 were trimmed to length ranging from 160-200 across sequencing runs. After merging, default settings
355 were used for error estimation and denoising with the following exception: `maxEE = 2`, meaning that
356 merged reads with expected error higher than `maxEE` were discarded (where $EE = \sum 10^{-Q/10}$).
357 Finally, after amplicon count inference, chimeras were removed and sequences of length 230-234
358 bp were retained; 37.5M (51%) reads passed filtering criteria. Taxonomic assignment of the 4,234
359 unique sequences was performed according to the Bioconductor workflow [10, 9] using RDP trainset
360 16 [63] and Silva v128 [51] databases.

361 **Phylogenetic tree estimation** In accordance with the Bioconductor workflow [9], two R pack-
362 ages were used to estimate a phylogenetic tree from obtained sequences. DECIPHER [66] was used
363 to perform multiple alignment, and `phangorn` [54] to fit a Generalized time-reversible Gamma rate
364 variation (GTR+G+I) maximum likelihood tree initialized at the neighbor-joining tree (parameters
365 `k=4` and `inv=0.2` were used for `phangorn::pml` function).

366 **Sequence clustering using phylogeny** Due to high resolution of the DADA2 pipeline, the ob-
367 tained amplicon sequence variant (ASV) count-matrix was very sparse. In order to attain more
368 overlap in organism counts between samples and subjects, we clustered ASVs into phylogenetically

369 similar sequence groups using the estimated phylogenetic tree. These clusters were generated by
370 cutting an associated phylogenetic tree at the height, $h = 0.1$, corresponding to median difference
371 between member sequences equal to 7.5 bp (out of 233 bp). This is equivalent to 96.8% sequence
372 identity, which correlates with thresholds to differentiate genera [5, 61]. We used both the original
373 ASV data and ASV-clusters for downstream analysis. This ASV-clustering approach is more accu-
374 rate than the traditional OTU clustering, as the phylogenetic distance is considered in the sequence
375 grouping process.

376 **Statistical Analyses**

377 **Ordination** To visualize the data we used Principal Coordinate Analysis (PCoA) with Bray-
378 Curtis distance applied to inverse-hyperbolic-sine (asinh) transformed count data. The transforma-
379 tion prevents the Bray-Curtis dissimilarity metric from placing too much weight on species/ASVs
380 highly abundant in all samples. All samples were included together in the computation of the
381 ordination projection. The plots were then faceted into distinct dietary intervention assignments.
382 Differences in community composition were tested using PERMANOVA (`adonis` function from the
383 `vegan` package [48], permutation = 999).

384 **Microbiota Diversity and Plasticity** Gut microbiota phylogenetic α -diversity was estimated
385 using the procedure of Nippress et al. [47], based on rarefaction curves. Phylogenetic diversity was
386 evaluated at a depth of 11,000 sequence reads – the level of the minimum library size of samples
387 post-filtering.

388 Daily pre-diet gut microbiota variability was estimated using pairwise β -diversity between each
389 subject's pre-diet samples. If a subject provided three samples, only the pairwise β -diversity for
390 consecutive days were used to compute the average. Consecutive samples in the discovery cohort
391 were taken a median of 1 day apart (IQR 1, 2) with an exception of a few subjects who provided
392 samples up to 5 days apart. The time span between an individual subject's samples was balanced
393 across weight-loss groups. To estimate variability between gut microbiota pre-diet and 10 weeks
394 into the dietary intervention, we calculated the pairwise β -diversity between each pre-diet and 10-

395 week sample for each subject, and then calculated the mean across all pairwise comparisons. For all
396 plasticity measures, Jaccard dissimilarity and unweighted UniFrac distance were used to assess the
397 variability in taxa presence/absence, whereas Bray-Curtis dissimilarity, weighted UniFrac distance,
398 and Jensen-Shannon divergence were used to estimate variability in abundances. The difference in
399 variability between VS and US subjects was tested for significance separately for each diet using
400 non-parametric Wilcoxon rank-sum test.

401 **Differential abundance testing** We looked for pre-diet ASV-clusters, and single ASVs, that
402 were differentially abundant with respect to 12-month weight loss. The inclusion of consecutive
403 daily samples (repeated measures) for subjects allowed for a more accurate estimation of the un-
404 derlying processes and helped reduce false discoveries. The `limma` differential abundance (DA)
405 testing framework [52] is the most conservative analysis that allowed us to model variability of
406 repeated measurements as within-subject random effects. Before using `limma` we transformed the
407 raw count data by first computing the library size factors using `estimateSizeFactors` from `DESeq2`
408 package [43] with the argument `type = "poscounts"`, which was specifically developed for sparse
409 sequencing data. We used a customized version of `limma::voom` function, where we substituted
410 the log2-counts per million (logCPM) transformation intended for bulk human RNA-seq data, with
411 an inverse-hyperbolic-sine ($\sinh^{-1}(x) = \log(x + \sqrt{1 + x^2})$) transformation shown to be more ap-
412 propriate for data which follows a negative binomial distribution (as is the case for 16S rRNA
413 gene sequencing) [40, 34, 7, 35]. Functions for differential abundance estimation from `limma` were
414 then used to fit the pre-diet ASV or ASV-cluster abundances. For increased modeling accuracy
415 and higher power, the method was applied to all samples from both diets together. Testing was
416 performed by evaluating the contrast between weight-loss success categories within each diet sepa-
417 rately (using diet-weight loss interaction terms in the model design). An additional fixed-effect term
418 corresponding to sample sequencing “lane” assignment was included in the model to account for
419 batch effects from different sequencing runs. For each of the two diets we tested both the contrast
420 between subjects from the VS compared to US weight-loss group, and also conducted secondary
421 analyses modeling the outcome as a continuous variable (percent weight lost at 12-months). Only

422 the taxa found in at least 10% of subjects on the given diet and significant after adjusting for
423 multiple hypothesis testing (Benjamini-Hochberg method [4]) at level $\alpha = 0.05$ were retained.

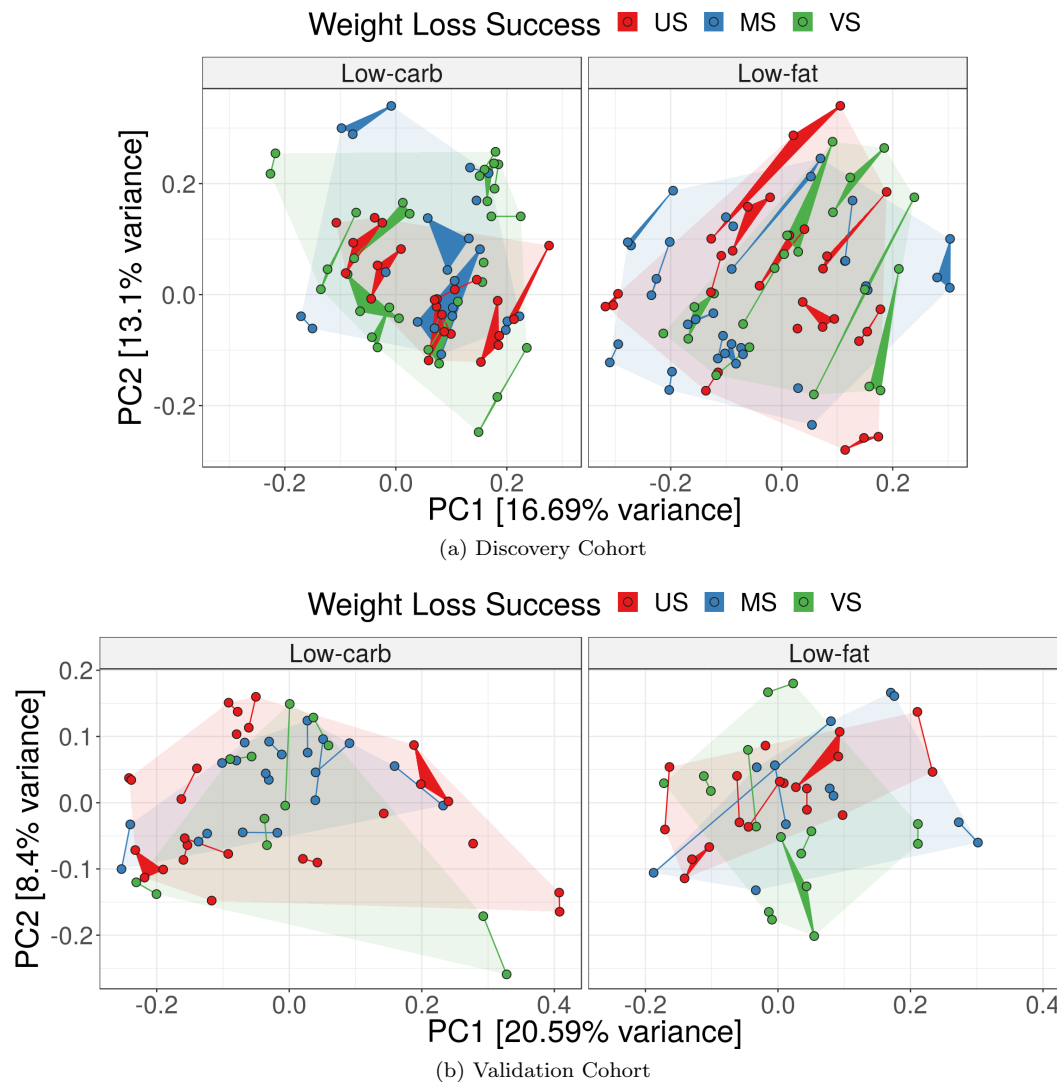


Figure 1: Pre-diet microbial community composition not correlated with weight-loss success. Pre-diet fecal microbiota collected from subjects in the discovery (a) and validation (b) cohorts prior to a low-carb (*left*) or low-fat (*right*) dietary intervention. Each point represents a single fecal sample and samples corresponding to the same subject are connected forming edges or triangles. Colors indicate 12-month weight-loss success: very successful (VS), moderately successful (MS), and unsuccessful (US). The faded background polygons show convex hulls for corresponding success categories. PCoA was computed with Bray-Curtis distance on inverse-hyperbolic-sine transformed counts.

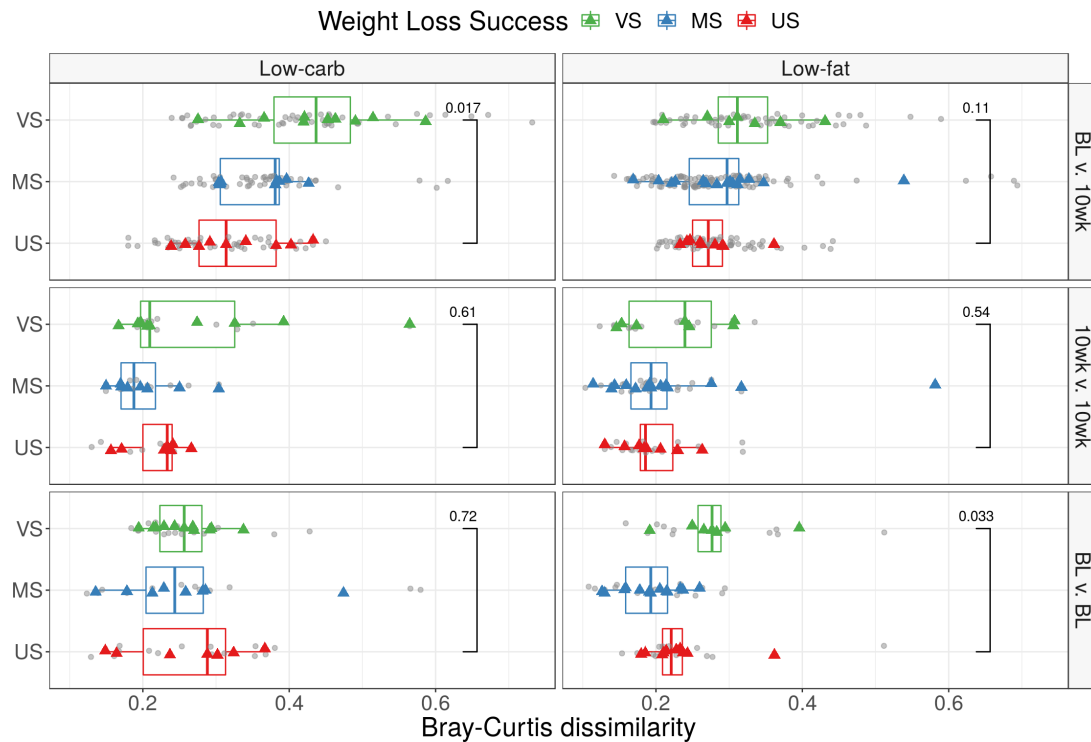


Figure 2: Gut microbiota plasticity over different periods. Pairwise β -diversity is shown between daily pre-diet samples (BL v. BL), between daily samples taken 10 weeks after initiation of the dietary intervention (10wk v. 10wk), and between BL and 10-week samples (BL v. 10wk). Bray-Curtis dissimilarities are shown for low-carb (left) and low-fat (right) diets. Grey points indicate computed pairwise dissimilarities between samples; colored points correspond to the average dissimilarity for each subject and are colored by weight-loss category: US – Unsuccessful, < 3% weight loss; MS – Moderately successful, 3 – 10% weight loss; VS – Very successful, > 10% weight loss. Results with other β -diversity metrics are shown in Figs. S3,S4

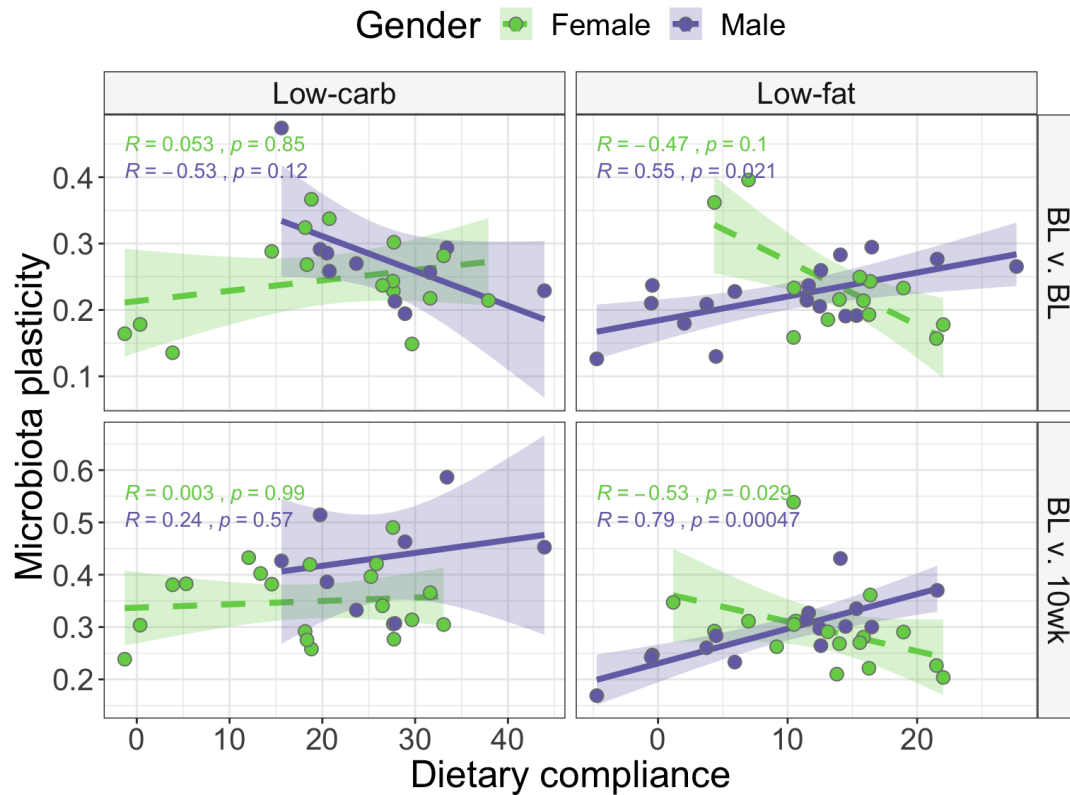


Figure 3: Gut microbiota plasticity correlated to dietary compliance in a sex- and diet-dependent manner. Spearman's rank correlations between dietary compliance and plasticity (measured as Bray-Curtis dissimilarity) between daily pre-diet samples (BL v. BL) and between pre-diet and 10-week samples (BL v. 10wk) are shown for low-carb (left) and low-fat (right) diets. Male (purple) and female (green) subjects show opposite correlations in many cases. Results with other β -diversity metrics are shown in Fig. S6.

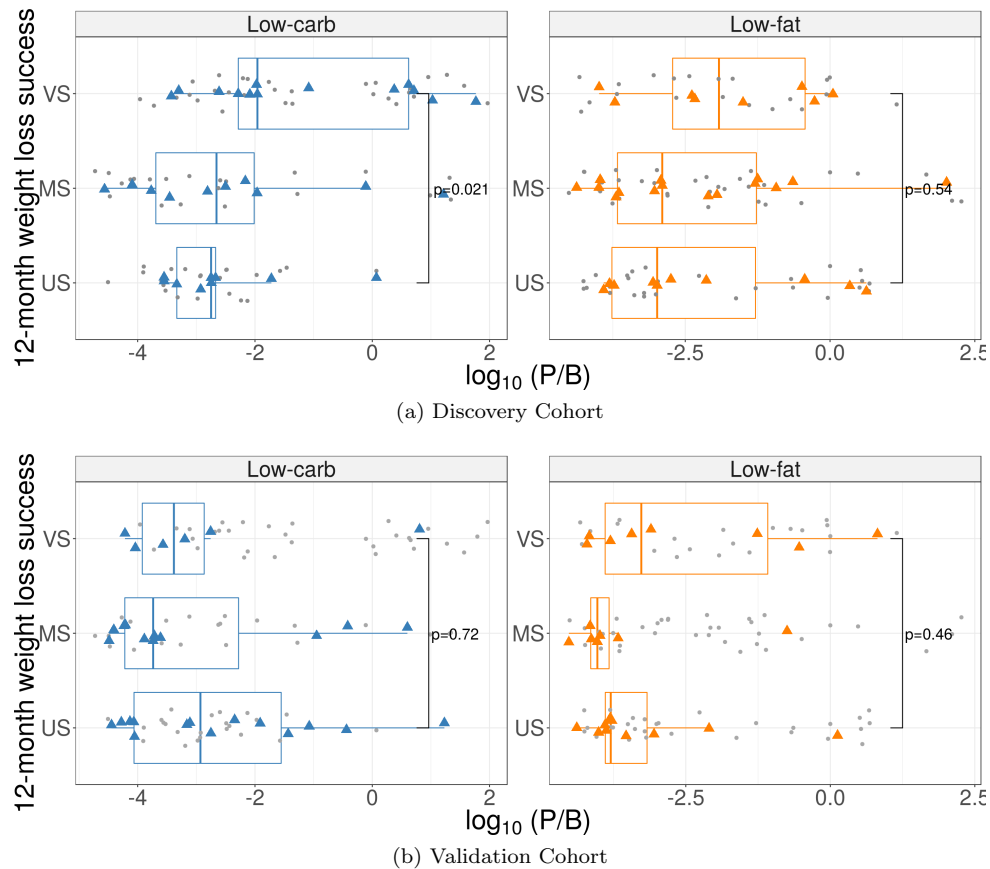


Figure 4: Differences in *Prevotella/Bacteroides* (P/B) ratio for subjects on a low-carb (left panels) or low-fat (right panels) diet. Grey points indicate P/B ratio for individual samples; colored points correspond to the average P/B ratio for each subject. Data from the discovery cohort (a) and the validation cohort (b) is displayed by subject's weight-loss success category at 12 months after the start of the dietary intervention: US – Unsuccessful, < 3% weight loss; MS – Moderately successful, 3–10% weight loss; VS – Very successful, > 10% weight loss. P-values shown for Wilcoxon rank-sum test comparing US and VS groups.

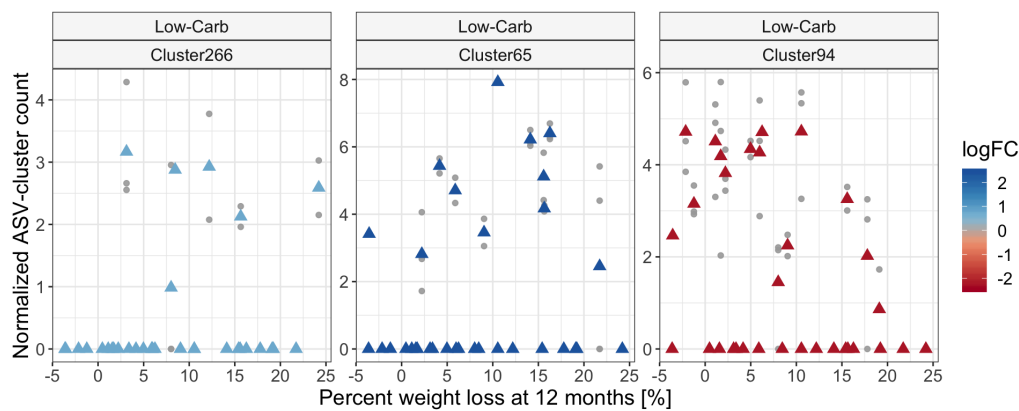


Figure 5: Taxa differentially abundant between weight loss success groups. ASV-clusters found differentially abundant when comparing subjects that were VS compared to US at 12-month weight loss on the low-carb diet. ASV-clusters were normalized and asinh-transformed for variance stabilization prior to analysis; the normalized, transformed values are shown on the y-axis. ASV-clusters have a median 96.8% sequence similarity (a taxonomic description can be found in Table 2). No taxa were found differentially abundant on the low-fat diet. Grey points represent individual samples and triangles represent the mean value for each subject.

Table 1: Subject characteristics and dietary intake for discovery and validation cohorts.

	width=1			
	Low-carb		Low-fat	
	Discovery (n = 32)	Validation (n = 31)	Discovery (n = 34)	Validation (n = 25)
Sex, n (%)				
Female	22 (68.8)	25 (80.6)	17 (50)	19 (76)
Male	10 (31.2)	6 (19.4)	17 (50)	6 (24)
Age, yr (SD)	43.1 (6)	42.5 (6)	40.5 (6.9)	39.6 (6)
Race/ethnicity, n (%)				
White	25 (78.1)	23 (74.2)	25 (73.5)	16 (64)
Hispanic	4 (12.5)	5 (16.1)	7 (20.6)	4 (16)
Asian	3 (9.4)	2 (6.5)	2 (5.9)	3 (12)
Other	0 (0)	1 (3.2)	0 (0)	2 (8)
Baseline weight, kg (SD)	92.9 (16.8)	90 (13.9)	96.1 (12.4)	92.6 (12.7)
Body mass index, kg/m ² (SD)	33.4 (3.7)	32.8 (3.5)	33.2 (3.3)	33.2 (3.5)
Weight loss ¹ , % (SD)	8.4 (7.7)	4.8 (6.2)	6.3 (7.7)	5.4 (7.6)
Weight loss ¹ success category, n (%)				
US	9 (28.1)	14 (45.2)	11 (32.4)	10 (40)
MS	10 (31.2)	11 (35.5)	15 (44.1)	7 (28)
VS	13 (40.6)	6 (19.4)	8 (23.5)	8 (32)
Dietary Intake				
Fat, % kcal				
Baseline	38.9 (6.1)	37.3 (6.4)	36.8 (6.5)	36.1 (5.2)
3 months	57 (9.1)	48.3 (8.7)	21.4 (7.2)	24.9 (7.5)
6 months	53.6 (9.1)	44.9 (7.5)	25.6 (7.6)	27.8 (9.1)
12 months	47.5 (10)	43.2 (8.4)	28.6 (8.2)	31.3 (7.9)
Carbohydrates, % kcal				
Baseline	44.6 (7.6)	45.9 (6.6)	46.4 (7)	46.8 (6.5)
3 months	17.6 (11)	27.7 (10.5)	59 (9.1)	54.4 (9.3)
6 months	22.1 (11.7)	31.4 (10.4)	55.6 (8)	52.3 (9.6)
12 months	27.3 (12.5)	35.4 (10.2)	53.3 (8.1)	48.1 (8.5)
Protein, % kcal				
Baseline	16.6 (3.4)	16.8 (2.9)	16.8 (3.4)	17.1 (3.4)
3 months	25.4 (6)	24 (6.1)	19.5 (4.6)	20.7 (5.4)
6 months	24.3 (5.2)	23.6 (6.8)	18.7 (4.2)	19.9 (5)
12 months	25.2 (7.1)	21.4 (4.9)	18.1 (3.9)	20.5 (4.5)
Dietary compliance ²				
Portion of diet from non-restricted foods ³ , % kcals (SD)	77.4 (10.3)	68.5 (9.1)	74.7 (5.7)	72.3 (7.4)
Reduction in restricted foods ⁴ , % kcals (SD)	21.9 (10.5)	14.4 (10.4)	11.4 (7.3)	8.4 (6.1)

¹ Measured after 12 months of dietary intervention

² Averaged over 3-, 6-, and 12-month dietary recall periods

³ Carbohydrate restriction for subjects on low-carb diet; fat restriction for subjects on low-fat diet

⁴ Decrease from baseline levels

Table 2: Log-fold changes of ASVs and ASV-clusters found differentially abundant when modeled against weight-loss success category at 12 months. Values represent log-fold changes between the US and VS groups. Bold values were significant for the cohort indicated. No clusters or ASVs were found differentially abundant in either cohort for the low-fat diet. * = $p < 0.05$, ** = $p < 0.001$

width=1					
ASV level	Cluster/Seq	Organisms Included	No. Seqs	Cohort	
				Discovery	Validation
	<i>low-carb</i>				
ASV-clusters	Cluster94	Ruminococcaceae_UCG-013 sp. & Clostridium_III sp.	10	-2.24**	0
	Cluster266	Enterorhabdus sp.	2	0.74*	-0
	Cluster65	Ruminococcaceae_UCG-014 sp.	69	2.45*	-1
	<i>low-carb</i>				
ASVs	Seq175	Ruminococcaceae_UCG-013 sp.	1	-2.19*	-0
	Seq64	Bacteroides sp.	1	-1.71*	0
	Seq77	Bifidobacterium sp.	1	-0.59	-2.3
	Seq169	Ruminococcus2 sp.	1	-0.25	-1.7
	Seq90	Faecalibacterium prausnitzii	1	0.48	2.7
	Seq746	Enterorhabdus sp.	1	0.88*	-0
	Seq182	Ruminococcaceae_UCG-002 sp.	1	1.30*	-0

Declarations

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Availability of data and materials

R scripts used for the analysis are available in Additional Files 1-6. All sequencing reads will be deposited in the Sequence Read Archive under BioProject number SUB5292808.

Author's contributions

JP and CDG conceived of and designed the project. TDH conducted all wet lab work. SH provided conceptual advice on analysis of data. JAG and LHN analyzed and interpreted the data. JP, CDG, SPH supervised research. JG prepared the manuscript, with contributions from all authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This research was approved by the Panel on Human Subjects in Medical Research (protocol 22035) at Stanford University, Stanford, CA, USA. All participants provided written informed consent.

Competing interests

The authors declare that they have no competing interests.

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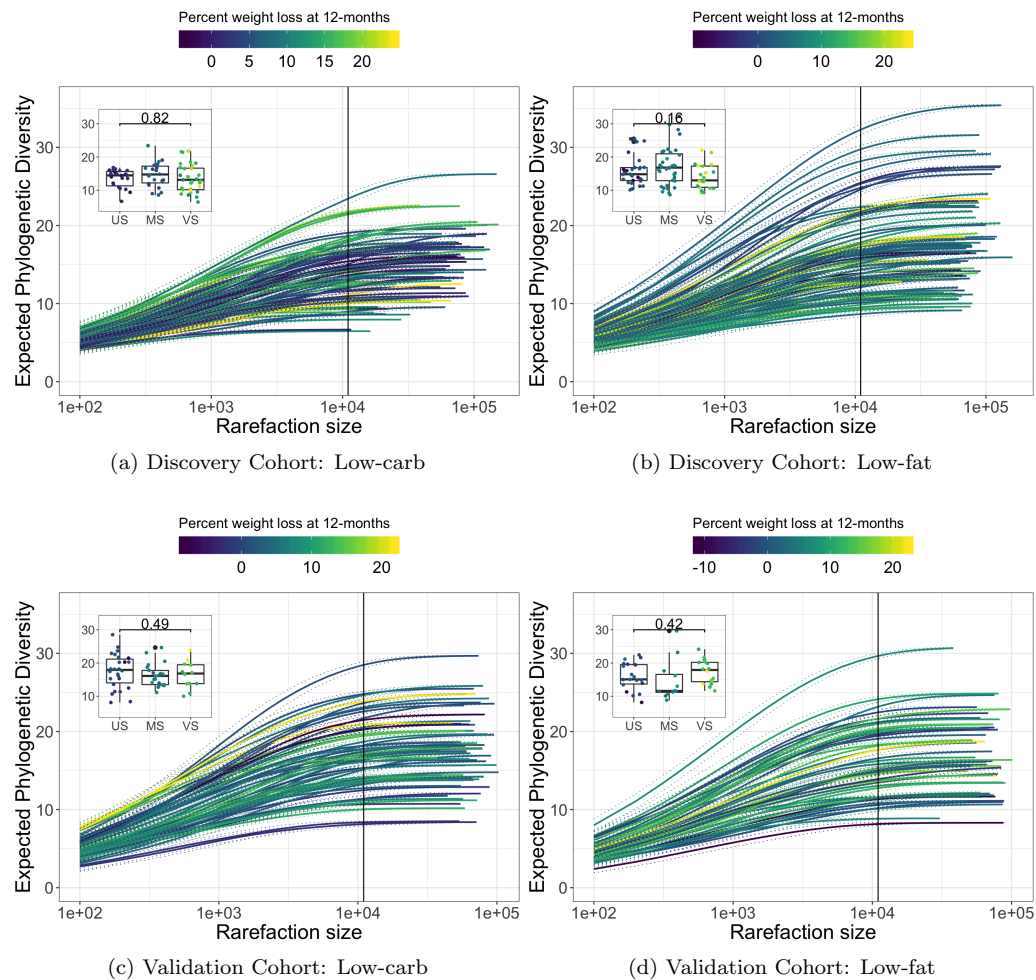


Figure S1: Phylogenetic α -diversity in pre-diet samples. Rarefaction curves for low-carb (*left*) and low-fat (*right*) diet, separated by discovery (*top*) and validation (*bottom*) cohorts. Curves were computed using the methods described in [47]. Inset boxplots report non-significant differences in α -diversity (at rarefaction level = 11,000 reads) between subjects from the VS and US 12-month weight loss success categories.

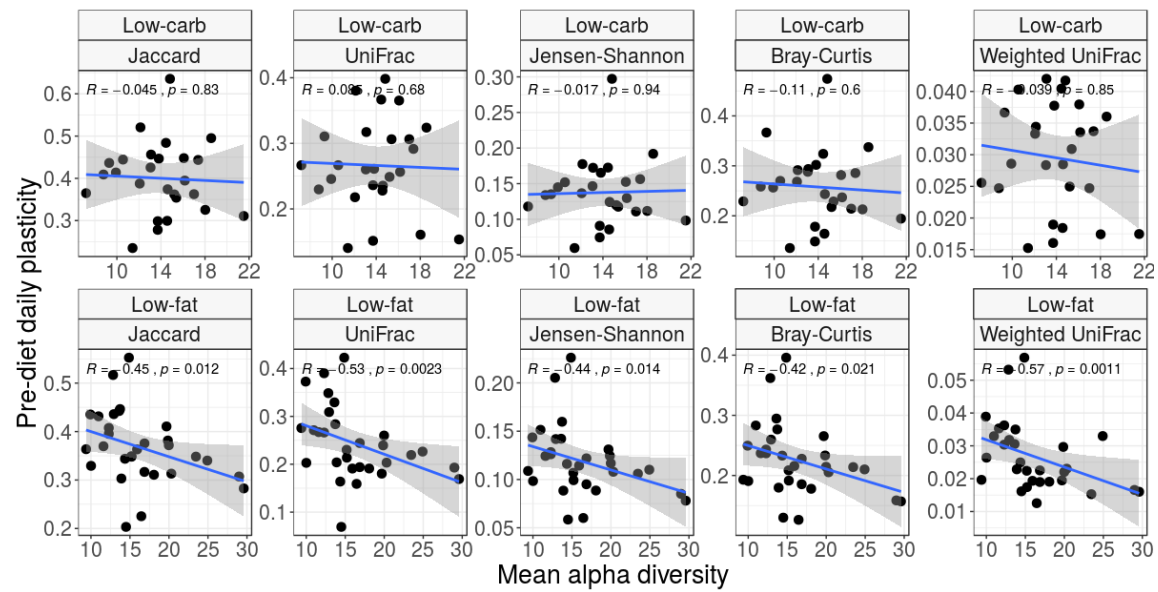


Figure S2: Beta diversity negatively correlated with alpha diversity. Mean baseline bacterial community diversity levels are negatively correlated with baseline bacterial plasticity for subjects on Low-fat diet.

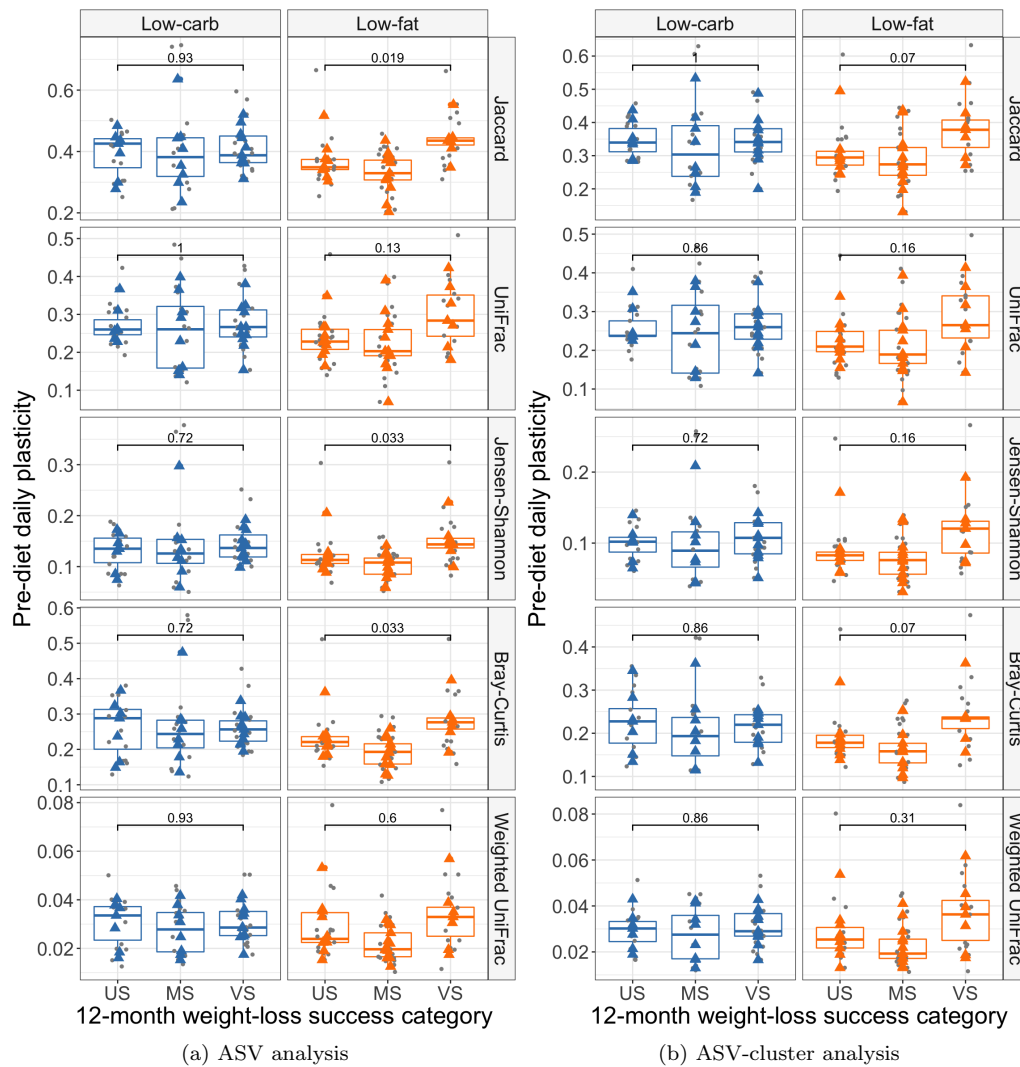


Figure S3: Day-to-day plasticity (β -diversity) between pre-diet fecal samples from subjects in the discovery cohort, grouped by 12-month weight loss success. Jaccard, unweighted UniFrac, Jensen-Shannon-Divergence, Bray-Curtis, and weighted UniFrac distances are shown for low-carb diet (left panels, in blue) and low-fat diet (right panels, in orange). Grey points indicate computed pairwise dissimilarities between samples; colored (low-carb and low-fat) points indicate the average sample-to-sample dissimilarity for each subject. US – Unsuccessful, < 3% weight loss; MS – Moderately successful, 3 – 10% weight loss; VS – Very successful, > 10% weight loss. P-values shown for Wilcoxon rank-sum test comparing US and VS groups. The analysis was done at the level of individual ASVs (a) and also ASV-clusters (b).

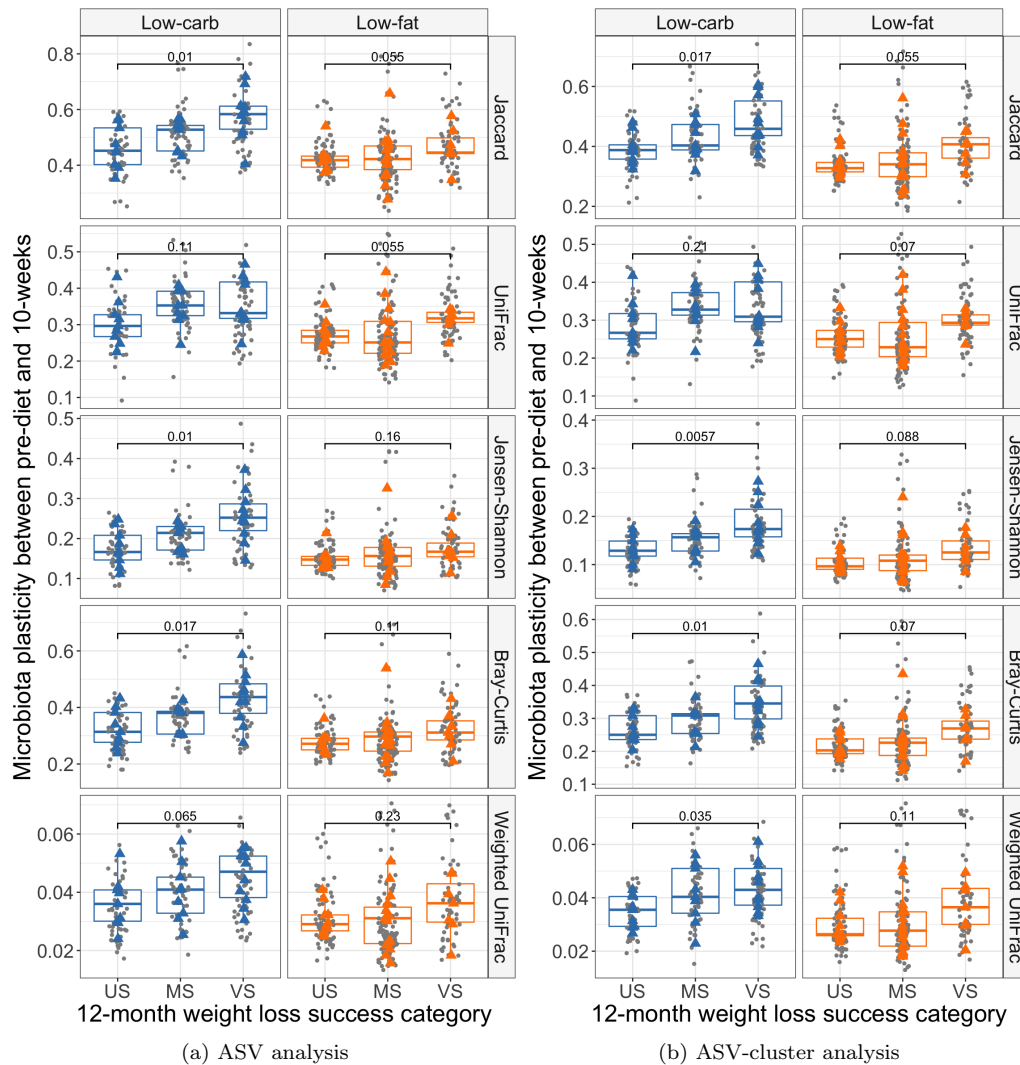


Figure S4: Microbial community composition shift over ten weeks, and in response to dietary intervention. β -diversity between pre-diet (baseline) and post-diet samples (taken at 10 weeks after initiation of the dietary intervention) from each subject was computed using Jaccard, unweighted UniFrac, Jensen-Shannon-Divergence, Bray-Curtis, and weighted UniFrac distances. Results shown for discovery cohort subjects on a low-carb (left panels, in blue) or low-fat (right panels, in orange) diet. Grey points indicate each computed pairwise dissimilarity between samples; colored points correspond to the average baseline-to-10-week plasticity for each subject. US – Unsuccessful, < 3% weight loss; MS – Moderately successful, 3-10% weight loss; VS – Very successful, \geq 10% weight loss. P-values shown for Wilcoxon rank-sum test comparing US and VS groups. The analysis was done at the level of individual ASVs (a) and also ASV-clusters (b).

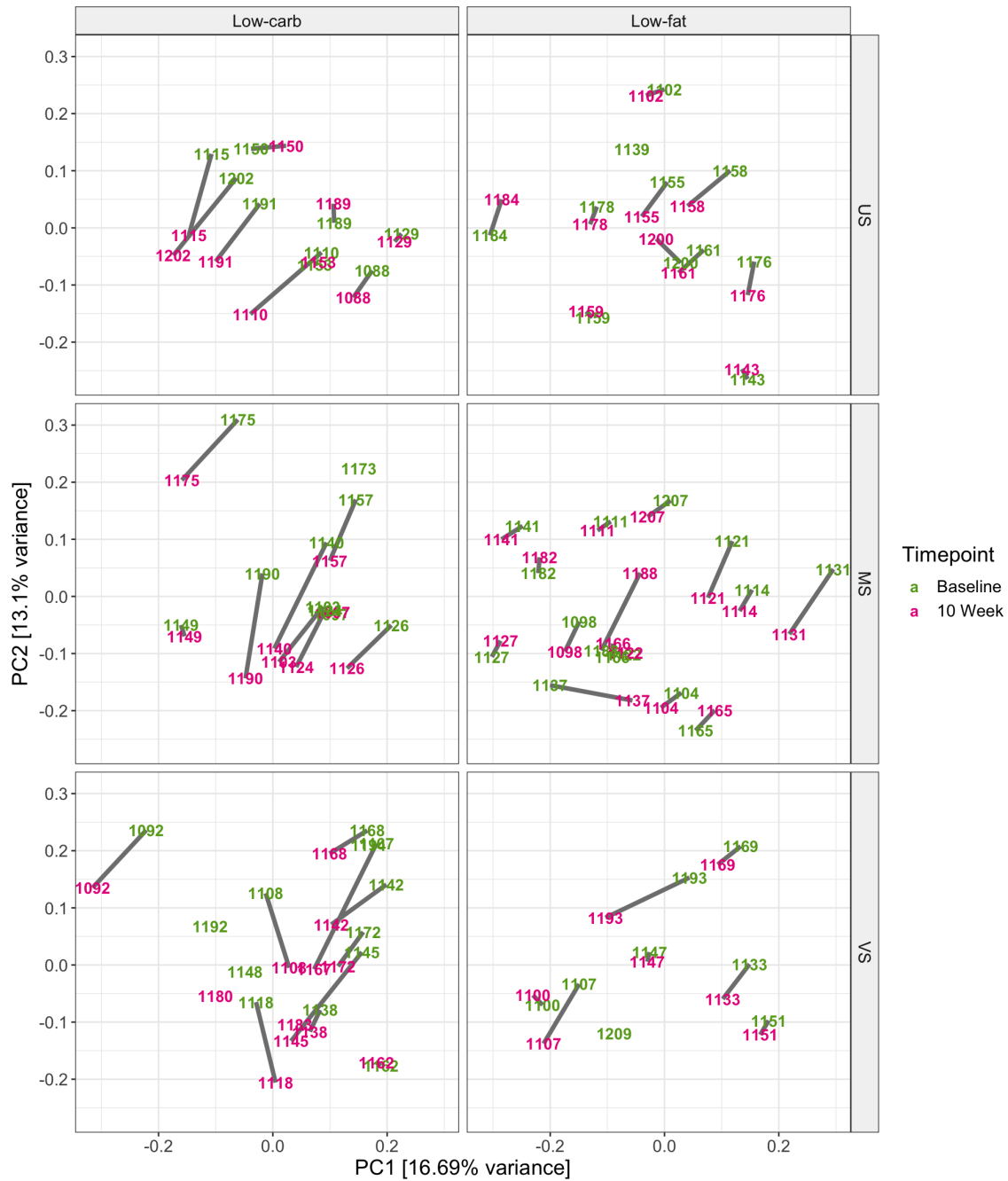


Figure S5: Microbiome shift in response to dietary intervention. Subject centroids (coordinates averaged over sample replicates) before (green) and 10 weeks after the start of the intervention (purple) for low-carb (*left*) and low-fat (*right*) diet. Data points are labeled with corresponding unique subject ID. Vertical panels correspond to weight loss success categories – very successful (VS), moderately successful (MS) and unsuccessful (US).

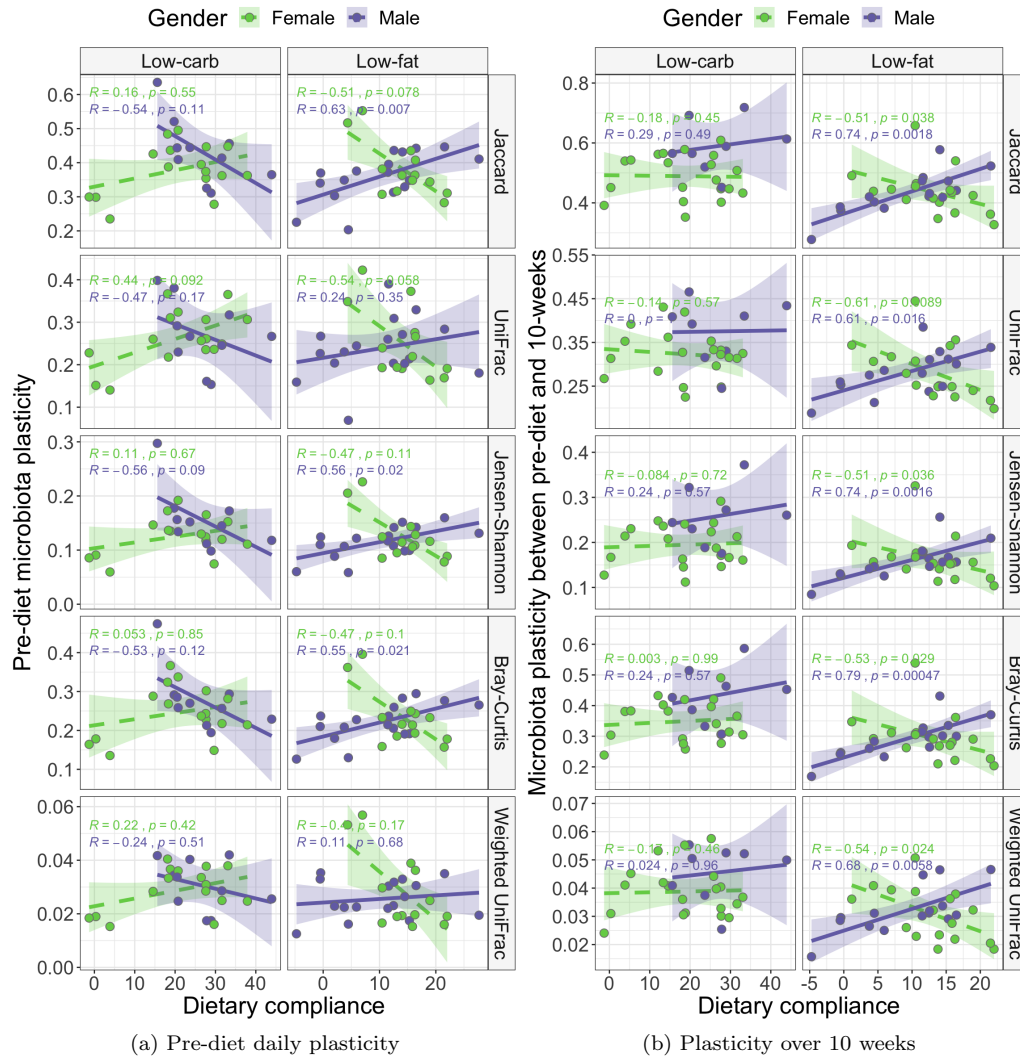


Figure S6: Sex- and diet-specific correlation between microbial community composition plasticity and dietary compliance. Plasticity across various β -diversity measures between daily pre-diet samples (a) and between pre-diet and 10-week samples (b) from each subject compared to dietary compliance. Results shown for discovery cohort subjects on a low-carb (left panels) or low-fat (right panels) diet. Male (purple) and female (green) subjects show opposite correlations in many cases. Spearman's rank correlation coefficients are shown for each subgroup.

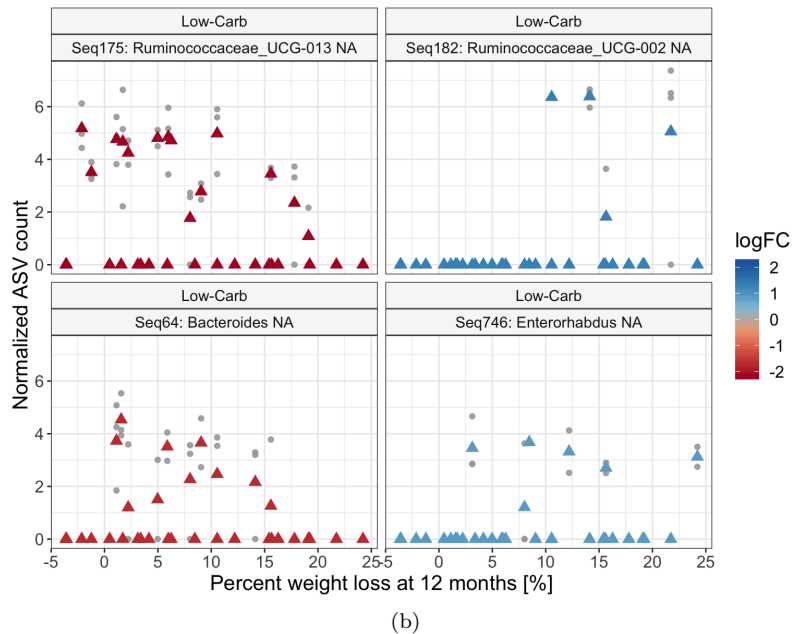
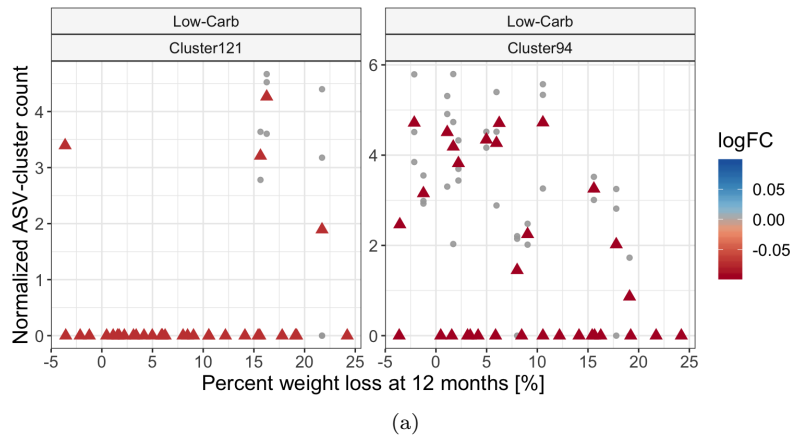


Figure S7: Taxa identified differentially abundant for subjects on low-carb diet. Analyses were conducted with ASV-clusters testing for difference in abundance across a continuous response variable – percent weight loss (a) and also with individual ASVs and tested for contrast between categorical weight-loss groups: VS compared to US subjects. Note: Cluster94 contains Seq175, as can be seen in the similarity of plots. Individual ASVs and ASV-clusters were normalized and asinh-transformed for variance stabilization prior to analysis; the normalized, transformed values are shown on the y-axis. Grey points represent individual samples and triangles represent the mean value for each subject. No taxa were found differentially abundant on the low-fat diet for either analysis.